Clues to understanding sudden infant death

– a role for *Helicobacter pylori* and innate immunity?

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1 Introduction

1.1 Sudden unexpected deaths in infancy

1.1.1 HISTORICAL ASPECTS

Early reference

The oldest mention of a sudden unexpected infant death dates back to about 500 B.C. and the story of King Solomons famous decision told in the First Book of Kings in the Old Testament: "This woman's child died in the night: because she overlaid it"[1]. Throughout the Middle Ages and well into the Reformation, overlaying was the accepted explanation of sudden infant deaths[2]. Regulated by King Christian V's Norwegian Law of 1687, the authorities were to solve whether or not the death was due to an accident or intentional infanticide[2]. If the overlaying was thought to be an accident, the punishment was pillory at the entrance of church followed by public confession. If considered manslaughter, the punishment was sentence by the secular court.

19th century: Status thymo-lymphaticus

In the late 1800s and early 1900s, sudden infant deaths were attributed to so-called "internal suffocation" induced by an abnormally large thymus. Designated "Status thymo-lymphaticus", the theoretical concept was that the enlarged thymus could compress the trachea and thus hinder the respiration[3]. The hypothesis was not rejected until the 1940s as it eventually became apparent that large thymuses indeed represented a normal developmental stage for infants at this age[4]. The misunderstanding clearly emphasizes the importance of appropriate controls in research on this subject.

1950s - Hypogammaglobulinemia

During the 1950s and 1960s, sudden unexplained deaths in infancy, mentioned as *crib deaths* or *cot deaths* attracted growing interest from pathologists and physicians. To spare parents the ordeal of an inquest which would necessarily follow an unnatural death, discrete morphological findings observed at autopsy were sometimes attributed to the cause of death[5]. Minimal inflammation of the lining of the larynx were observed in several cases and promoted the hypothesis that viral infections could be a causative factor[4]. However, attempts to demonstrate evidence of viremia in victims of crib death failed.

In 1954, it was purported that crib deaths could be due to hypogammaglobulinemia, as low levels of serum gammaglobulin were found in victims of crib death compared to standards available at that time, which were normal adult values[6]. Valdes-Dapena demonstrated a few years later that the serum levels in victims of crib death did not differ from live infants at the same age[7]. Other theories of causative factors to crib death evolving in this period were: Hypersensitivity to cow's milk, hypoparathyroidsim, and "reshaping" of myocardial conduction system within the first month of life[4]. All these theories were rejected by subsequent research.

Defining SIDS

Lack of uniform clinical terminology created difficulties for cilinicians and researchers as well as parents of deceased infants. Parents were desperate for a definition that could dispel

unwarranted suspicion of child abuse and provide the medical basis for appropriate counseling[8]. In 1963 and 1969 the National Institute of Child Health and Human Development (NICHD) arranged international conferences on the causes of sudden death in infants. A consensus was made in defining the phenomenon of unexplained infant deaths by the term *Sudden Infant Death Syndrome* (SIDS)[9]. Formulated by Beckwith, SIDS was defined as:

"*The sudden death of any infant or young child, which is unexpected by history, and in which a thorough post mortem examination fails to demonstrate an adequate cause of death*".

The definition emphasized the necessity of an autopsy to classify sudden infant deaths. A degree of diagnostic stability and focused research was achieved with this definition, as well as improved ability to compare statistics from different regions and countries.

The present (San Diego) definition[10], approved at the SIDS International Conference in Edmonton, Canada in 2004, was based on a proposal by Beckwith for a new SIDS definition which he presented in Sidney 1992[11]. According to this definition, SIDS is:

"The sudden unexpected death of an infant <1 year of age, with onset of the fatal episode apparently occurring during sleep, that remains unexplained after a thorough investigation, including performance of a complete autopsy and review of the circumstances of death and the clinical history."

The San Diego definition provides a number of specifications for the diagnosis (i.e. age criteria, type of investigations, interpretation of findings) used for subclassification into category I ("pure") SIDS or category II SIDS. Criteria for category I SIDS include: age between the third week and ninth month, a normal clinical history including a full term pregnancy and no evidence of accidental death in the sleeping environment (i.e. bed-sharing or prone sleeping on soft surface). Fully investigated, yet totally unexplained infant deaths that do not meet the strict requirements are placed in a category II SIDS, to some extent corresponding to the borderline term in the Nordic protocol[I2]. The San Diego definition also introduces the term "Unclassified" sudden infant death, referring to cases incompletely investigated or that do not meet the criteria for category I or II SIDS, but for which alternative diagnoses of natural and unnatural conditions are equivocal.

1970s: SIDS mortality rates increasing

During the 70s and 80s the SIDS rates in several western countries were rising, instigating increased awareness from researchers[13]. Several experimental studies were initiated in order to try to explain the enigma. Different theories of explanations were put forward, some were disputed and others seemed hopeful, yet at the time unable to fully explain the concept. Proposed theories included that SIDS was due to neurological malformations, heart arrhythmias, circadian autonomic disturbances, infection, immunological disturbances, endocrine or metabolic disorders as well as extrinsic causes: overlaying, suffocation, poisoning. A selection of these theories is presented below:

Steinschneider brought forward the apnea hypothesis[14] based on the documentation that episodes of apnea preceded several cases of SIDS. The hypothesis led to the development of apnea monitors for the use of parents in order to be alarmed if theirs babies stopped breathing. The use of monitors and the apnea theory were brought into miscredit as it later

appeared that two of the infants described by Steinscheider as dying suddenly and unexpectedly in fact were homicides.

The heart arrhythmia theory was fronted by Schwartz[15]. He argued that ECG screening had the potential to save infants from dying of SIDS[15]. His group later performed ECG of 33000 newborn infants, 24 of which ended up dying of SIDS[16]. Long QT interval was detected in 12 of these 24 ECG diagrams. (The heart arrhythmia hypothesis has recently been "confirmed" by studies performed in collaboration with our research group in Oslo, documenting mutations or polymorphisms in the Long QT genes in 9.5% of SIDS victims and none of the controls[17]).

Kahn proposed that autonomic dysfunction or impairment could be the cause of SIDS[18]. Infants who had been studied and subsequently died from SIDS, compared with surviving infants, had higher baseline heart rates, lower heart rate variability, and imbalance in parasympathetic/sympathetic tone[16;19-21].

In 1976, Naeye presented findings of brain stem astrogliosis and six other tissue markers for chronic or repeated hypoxia in sudden infant death[22]. The brain stem findings were reproduced by several other investigators[23-26]. However, astrogliosis takes several days (weeks) to develop and probably represents status subsequent to hypoxic episodes prior to death, i.e. in fetal life. The hypoxia theory gained support when Rognum and Saugstad in 1988 presented their studies on hypoxanthine, the breakdown product of purine metabolism[27]. They found elevated hypoxanthine levels in vitreous humor in SIDS compared to violent deaths, which indicated that a proportion of the SIDS victims probably had sustained episodes of hypoxia in close proximity to the time of death.

Break-through – the hazard of prone sleeping

In the early 70s, Dr Shirley Tonkin in New Zealand, Dr Susan Beal in Australia and Dr DeJonge in the Netherlands visited each family that had lost an infant and learnt that the main common feature was that the babies had slept prone[28-30]. The observations triggered large case control studies which confirmed the relationship between prone sleeping position and SIDS[31;32] The back-to-sleep campaigns of the late 80s and early 90s led to a dramatic drop in infant mortality rates around the world[33-35]. The change of infants' sleeping position from prone to back represents the success story of SIDS research. Norway was one of the first countries to implement the back-to-sleep campaign, induced by the effort of Dr. Markestad in Bergen in late 1989. The incidence in Norway dropped from 142 cases of SIDS (2.4 ‰ of all live births) in 1989 to 34 cases three years later (0.6 ‰), and has continued to decline to around 20 cases per year after the millennium (0.3-0.4 ‰, Figure 1) (www.ssb.no).

SIDS research in the 80s and 90s was dominated by epidemiological studies. Demographic and individual risk factors for SIDS were detected and reproduced in different populations in large multi-center studies. The most important risk factors are presented in section 1.1.3 below.

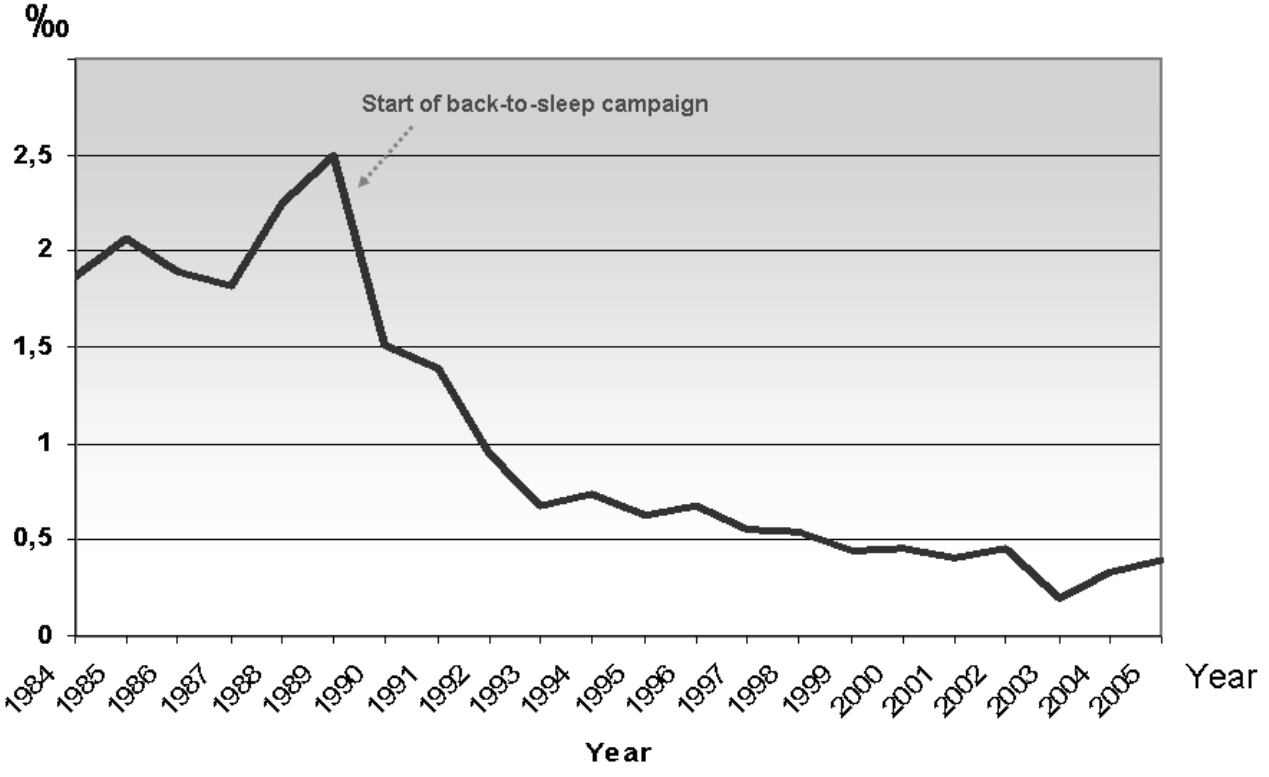

Figure 1. The SIDS rate (No of deaths pr 1000 live births) in Norway 1984-2005

The Fatal triangle hypothesis

In 1992-93 three analogous theories for the explanation of SIDS was presented by different research milieus, although mentioned by Wedgewood already in 1972[11;36]. These theories imply that for an infant to die of SIDS, three conditions must be fulfilled:

1. A predisposition (i.e. inborn genetic error or intrauterine exposure to nicotine)
2. A vulnerable developmental stage (i.e. the first months of life) and
3. An exogenous trigger event (prone sleeping, infection, overheating).

The three-hit models proved helpful in order to relate epidemiological and biochemical research. Experimental studies as well as studies of the mucosal immune system in SIDS victims and controls during the last 20 years have generated knowledge and theories of possible predisposition and features of the vulnerable stage in infancy important for the understanding of SIDS[13;37]. The Fatal triangle hypothesis (Figure 2) will be further addressed in the general discussion 5.1 below.

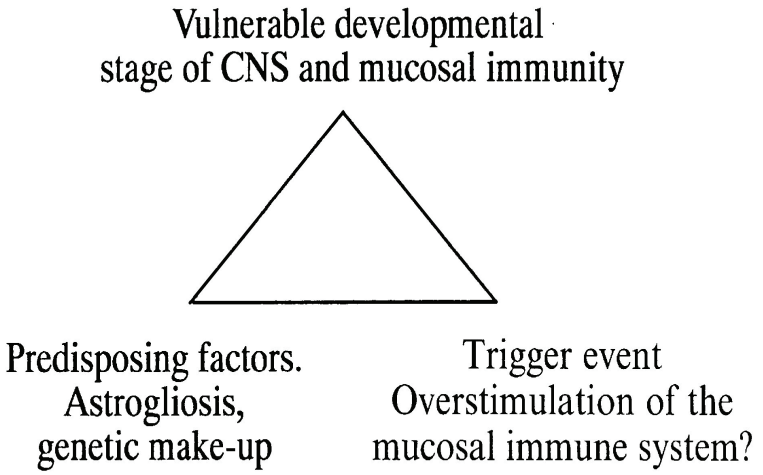

Figure 2. The Fatal triangle model explaining SIDS. From Rognum and Saugstad[38].

1.1.2 Diagnostic challenges – what is unexplained and what is explained

As part of the NORD SIDS study in the early 90s, forensic pathologists from the Nordic countries worked together to establish a protocol for the investigation of sudden infant death[39;40]. The Nordic protocol divided sudden unexpected infant deaths into three categories: pure SIDS, borderline SIDS and non-SIDS. The pure SIDS cases were totally unexplainable and the non-SIDS cases explained deaths. In between the fully explained and

totally unexplained deaths, the borderline SIDS diagnosis referred to cases of sudden infant death in which pre-existing congenital disorders or clinical symptoms, and/or post-mortem findings were present, but not considered severe enough to explain the cause of death. Definite pathologic criteria for diagnostic classification were agreed upon, and supplemented in all the Nordic countries.

Several conditions involving all organ systems may be responsible for sudden death in infancy[41]. If an adequate post mortem examination is not performed, including a forensic autopsy, death scene investigation and review of the history, the possibility of determining the cause of death may be lost[11]. Evidence of neglect, abuse and homicide can be subtle, and inflicted asphyxia may be indistinguishable from SIDS[42]. Before 1989, approximately 80% of all sudden deaths in infancy remained unexplained, after the post-mortem investigation categorized as SIDS or Borderline SIDS (Figure 3).

After the decline in SIDS rate, the relative percentage of explained deaths has increased, in particular deaths due to diseases and accidents. Moreover, in later years several cases of infant deaths due to neglect and child abuse have also been disclosed (Figure 3). These changes are not likely to be due to a shift in diagnostic evaluation, as shown by a reevaluation of the cases examined prior to 1989, classifying the cases according to the Nordic criteria of 1992[43]. The "new" manifestation of sudden infant death, represent a tremendous challenge for the medical experts and police involved in such investigations and a standardized approach to the autopsy and death scene investigation is necessary.

Ruling out inflicted deaths may have serious medicolegal implications. Efforts have been made to reach agreement on standardized international protocols explaining the baseline investigations that need to be performed[41;44]. The baseline forensic autopsy of sudden deaths in infancy includes radiological, microbiological and toxicological investigations, neuropathological examination, histology of all organs and targeted genetic screening[45;46].

International agreement has been reached as to when specific pathological changes may constitute the cause of death[41;45]. How to interpret information obtained from clinical history and from death scene investigation is under current discussion[12;47]. The SIDS definition emphasizes the necessity of death scene investigations to exclude other causes of death[10]. In my opinion, a prerequisite for classification of sudden infant deaths are multi-disciplinary case conferences where circumstantial, clinical and pathological findings are being evaluated[47;48]. In Norway, death scene investigations are not mandatory, and in general not performed, unless the autopsy reveals suspicion of a possibly inflicted death. During a research project in South east Norway between 2001 and 2004 however, we were able to carry out such surveys[48]. The importance of examining the death scene was shown by the fact that in 31% of the cases in the research period, the death scene investigations were of value for the diagnosis[48].

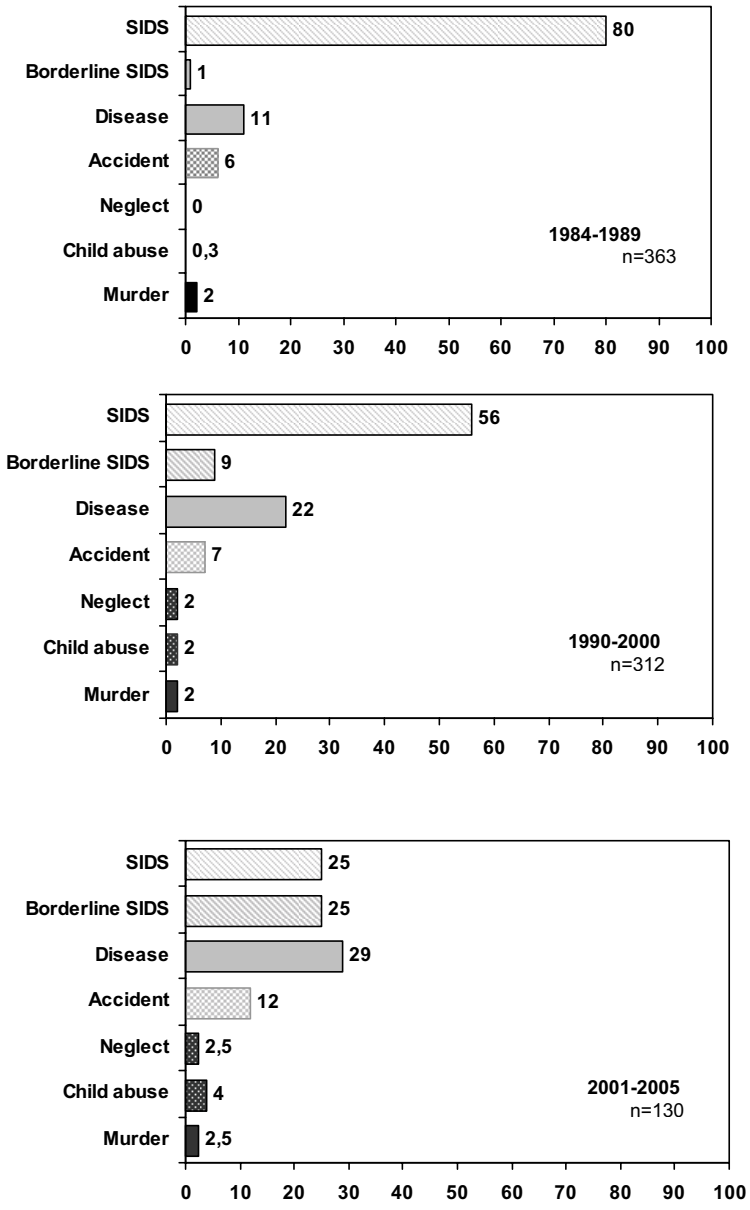

Figure 3. Relative percentage of unexplained and explained sudden infant deaths in Southeast Norway in three time periods, 1984-89 (before the back-to-sleep campaign), 1990-2000 and 2001-05.

1.1.3 SIDS EPIDEMIOLOGY

Demographic factors

Boys are more likely to die from SIDS than girls at a ratio of 3:2[49;50]. SIDS is also associated with prematurity and low birthweight as well as low maternal age[50;51]. Before the drop in SIDS rate, most of the victims were aged between 2 and 4 months[52]. The back-to-sleep campaign decreased and broadened this classical age peak. Approximately 60-65% of the sudden infant deaths (SIDS and explained deaths) occur during the winter months from October to March, but the seasonal variation has become less apparent after the drop in SIDS rate[51]. With the decreasing rate, socio-economic factors have become more obviously associated with SIDS. Blair et al found that prior to 1991, 28% of the SIDS parents were unemployed, compared to 48% after that year[49]. On the other hand, the proportion of males/females has remained consistent[49;51].

It has been argued that breastfeeding has a protective effect, but no significant effect of breast- /bottle feeding has been shown in epidemiological studies [50;51;53]. Siblings of SIDS victims are at increased risk for SIDS[50], which may be attributed to unrecognized genetic predisposition and/or to common environmental factors, however, homicide must in such cases also be ruled out.

Prone sleeping position

The rate of placing infants prone for sleep has decreased substantially after the successful campaigns. However, more than 50% of infants older than 2 months of age are still found dead in the prone position[54]. The dangers of prone sleeping position have been attributed to the possibility of rebreathing of expired air, which contains high levels of carbon dioxide[55]. Some infants with blunted arousal systems may fail to turn their head or lift their face[56]. Prone sleeping infants with fever are particularly at risk[57]. The prone position raises the upper airway surface temperature and an increased risk for bacterial toxin production has been hypothesized[58]. Prone sleeping has also been shown to increase the total amount of time infants spend asleep and, in particular, the time spent in quiet sleep, a state of reduced arousability [21;59].

Smoking

The most important environmental risk factor after prone sleeping is smoking during pregnancy[60]. Prenatal exposure to tobacco smoke affects fetal growth and is associated with increased risk for prematurity and low birth weight[61] and delayed lung growth[62]. Functional impairment of the respiratory response to hypoxia has also been shown in infants exposed to tobacco smoke in utero[63]. Post-natal exposure to tobacco smoke also seems to be a separate risk factor for SIDS[60]. High levels of the nicotine breakdown-product cotinine has been detected in SIDS victims[64], suggesting that exposure to nicotine has occurred within the last hours prior to death. Nicotine has a direct inhibitory effect on neurologic development and is associated with decreased arousal to hypoxia[65] and alterations in the autonomic control[66].

Bed-sharing

In the aftermaths of the back-to-sleep campaign several studies have shown an increased risk for sudden infant death associated with adult-infant bed sharing. In 2005 we presented data demonstrating that the SIDS rate in infants below two months of age has not been

altered by the back-to-sleep campaign, and that 73% of SIDS victims in this age group were found dead in a bed-sharing situation[54]. Bed-sharing may be hazardous when the infant is younger than 4 months of age and the bed-sharing parent is a smoker or is influenced by drugs or alcohol[50;54;67]. On the other hand, safe bed-sharing is likely to facilitate breastfeeding and enhance parent-infant interactions[68;69], and our study disclosed that bed-sharing tended to be more common in controls than in SIDS victims for infants older than 4 months of age.

Soft bedding and overheating

Soft bedding and soft surfaces, including pillows, sheepskins and porous mattresses have been associated with higher risk for SIDS[56;57]. In particular, a strong interaction has been found between prone sleep position and soft bedding surface[57]. During infancy the metabolic activity increases substantially and the ratio of body surface to mass decreases. Infants show a very wide range of metabolic activity and heat production during sleep[70;71]. The main factor likely to compromise thermoregulation, with increased risk of SIDS, is head covering[72]. It has been argued that the risk of overheating could possibly be reduced by the use of an infant sleeping bag[72].Some of the "classical" risk factors are not merely restricted to cases of SIDS, but are also associated with deaths due to infectious disease[49] (Table 1).

Table 1. Comparison of epidemiological variables in SIDS, Borderline SIDS, deaths due to infections and accidental/inflicted deaths below 1 year of age investigated at the Institute of forensic medicine in Oslo between 1984 and 2005.

	SIDS n=208	**Borderline SIDS** n=64	**Deaths due to infections** n=42	**Accidental/ inflicted deaths** n=21
Median age in days (IQR)	103 (84)	89 (98)	88 (131)	176 (219)
Winter occurence	61 %	56 %	55 %	52 %
Male predominance	56 %	61 %	69 %	48 %
Prone sleeping*	72 %	57 %	62 %	
Bed-sharing infants < 2 mo of age#	57 %	52 %	57 %	

*Data regarding sleeping position was present in 180/208 SIDS, 52/64 Borderline SIDS and 34/42 deaths due to infections.

#Data regarding occurrence of bed-sharing or not was present in 42/43 SIDS, 23/25 Borderline SIDS and 14/15 deaths due to infections below 2 months of age.

1.1.4 SIDS AND INFECTION

More than 60 years ago, Werne hypothesized that an ordinary seasonal respiratory infection may cause the death of an otherwise healthy infant[73]. Prior to the back-to-sleep campaign more than half of SIDS victims reportedly had a mild infection during the week preceding death[52]. In fact, for the "classical" SIDS victims between 2 and 4 months of age, approximately 2/3 had recently underwent a slight infection and interestingly, this population subgroup was most affected by the back-to-sleep campaign [52]. The typical winter peak in SIDS rate corresponds with endemics of respiratory infections. Lindgren et al demonstrated a significant correlation between outbreaks of whooping cough due to *B. Pertussis* infections and SIDS mortality rate[74]. It has also been postulated that the SIDS rate is related to outbreaks of respiratory virus epidemics [75-77](Figure 6 in section 5.1.3 below).

The hazards of sleeping prone have also been attributed to increased risk for infections. The prone position raises the upper airway surface temperature, which enhances the growth conditions for pathogenic bacteria such as *Staphylococci* and *Neisseriae*[58]. *Staphylococci* toxins and endotoxins from enteric bacteria such as *Clostridium* and *E. Coli* have been suggested as contributory factors to SIDS, inducing shocklike reactions and fever[78]. Endotoxins have been detected in individual SIDS cases, but have not been investigated in large case-control studies[79].

1.1.5 SIDS AND THE IMMUNE SYSTEM

Mild inflammatory changes in the wall of the bronchioli in SIDS victims was demonstrated by Paltauf in the late 1800s[3]. During the last 20 years, extensive immunohistochemical examination of the mucosal immune system in SIDS has been performed on tissue samples form the SIDS Biobank of the Institute of Forensic Medicine at the University of Oslo. Salivary glands, tonsils, tracheal walls, and intestinal mucosa have all shown immune stimulation in SIDS, though to a somewhat lesser degree than in victims of infectious deaths[80-83]. The findings are summarized in figure 4.

The newborn infant is immunologically naive, as the acquired immune system is dependent upon interaction with microbial antigens. Studies have demonstrated that the acquired mucosal immune system undergoes a rapid development in the first week and months after birth[84-86]. In contrast, the amount of secreted anti-microbial enzymes such as amylase, lysozyme and lactoferrin decreases shortly after delivery[84]. Furthermore, in the first weeks and months after birth, the amount of circulating maternal immunoglobulins from fetal life drops. This obvious imbalance in mucosal immunity probably reflects a vulnerable period of life. It has been postulated that components of the innate immunity in this age period play an important role in the protection against infections[87;88].

A large proportion of SIDS is probably preceded by repeated hypoxia, gasping and bradycardia shortly prior to death[13]. The downregulation of respiration is most likely due to events in the central nervous system. In 1989 Guntherroth proposed that cytokines might constitute a link between activation in mucosal (peripheral) immunity and the central nervous system[89]. In 1995 Vege et al demonstrated that half of the SIDS victims had interleukin 6 (IL-6) levels in cerebrospinal fluid in the same range as infants who died from infections such as meningitis and septicemia[90]. IL-6 is a pro-inflammatory cytokine mediating fever and an acute phase response. In a later study, it was demonstrated that SIDS victims with high IL-6 levels in the cerebrospinal fluid also had slight signs of infection before death, and showed increased number of either IgA cells or HLA DR

expression in the laryngeal epithelium[82]. An Australian study on IL-6 gene polymorphisms revealed that a genotype associated with high IL-6 responses was more frequent in SIDS victims compared to controls[91]. However, no such association was found in the Norwegian population[92]. Interleukin 10 (IL-10), an anti-inflammatory cytokine, has also been implicated in SIDS: IL-10 gene polymorphisms were found to be associated with sudden deaths due to infections[93]. SIDS took an intermediate position between deaths due to infections and controls.

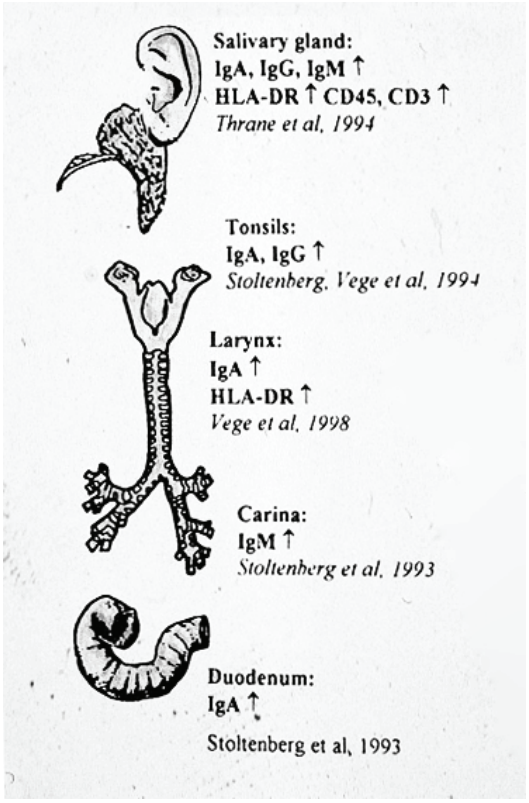

Figure 4. Summary of the immunological and biochemical findings in material from the Institute of Forensic Medicine, University of Oslo.

1.2 Helicobacter pylori

In 1983 Warren and Marshall described the growth of a spiral shaped bacilli in biopsy specimen from the gastric mucosa[94]. Soon after the bacterium was linked with chronic antral gastritis and peptic ulceration[94]. Initially, the bacterium was classified as *Campylobacter pylori* but in 1989 was included in a new genus, *Helicobacter,* and renamed *Helicobacter pylori*[95]. *H. pylori* is a S-shaped or curved gram-negative rod. The principal reservoir for *H. pylori* infection appears to be the human stomach, especially the antrum.

1.2.1 Prevalence of *H. pylori* infection

H. pylori is a common bacterium and approximately 50% of the world's population has been estimated to be infected[96]. In most western countries the prevalence in the adult population is between 20-40% compared to 70-85% in developing countries. The increased prevalence with age is largely due to an age-cohort effect rather than new infections. Colonization typically takes place in early childhood and it has long been assumed that the encounter with bacteria results in a lifelong infection. In the Nordic countries the prevalence in children is presumably low, between 2-5% in pre-school children[97;98].

1.2.2 Transmission and colonization

When *H. pylori* is introduced in the human stomach, it may pass through to the intestine or it may colonize the gastric mucosa[99]. After colonizing the stomach, *H. pylori* either causes an acute infection or is spontaneously eliminated. In children such a spontaneous eradication can occur several times before colonization takes place[100]. Whether this depends on the infectious dose of a given *H. pylori* strain or results from exposure to several *H. pylori* strains with different characteristics is unknown.

The route of transmission is not totally clarified, however is most likely fecal-oral or oral-oral[101]. Person-to-person transmission within the family appears to be the predominant mode of transmission, particularly from mothers to children and among siblings, indicating that intimate contact is important[102]. There is now emerging evidence that acquisition of *Helicobacter pylori* infection in childhood does not necessarily result in persistent infection[103;104].

The ability of *H. pylori* to colonize and establish infection in the gastric mucosa is dependent upon some basic characteristics: urease, flagellae, a particular shape, and adhesins[105]. The bacteria produces urease which converts urea to ammonia ions that neutralize the acidic gastric juice, enabling *H. pylori* to survive and multiply in the stomach. Colonizing the mucin layer that covers the epithelial cells, the flagellae give *H. pylori* the mobility to withstand rhythmic gastric contractions and penetrate the gastric mucosa. The curved s-shape and flagellae both enables the bacteria to bore through the mucin layer, and due to production of specific adhesion molecules the bacteria is able to adhere to gastric epithelial cells.

Recently, it has been recognized that *H. pylori* has the ability to convert into a coccoid form when exposed to detrimental environmental circumstances [106]. Clustered coccoid bacteria were visualized in biopsies from the gastric mucosa of dyspeptic patients by electron microscopy. The biopsies were PCR-positive, yet culture-negative, and seemingly void of a host immune response [107].

1.2.3 The immunological response

H. pylori releases large amounts of small water soluble antigens eliciting the host epithelial cell production of chemokines and pro-inflammatory cytokines such as IL-8 and IL-6[105]. Recent studies have shown that innate components with the ability to recognize pathogen-associated molecular patterns i.e. Toll Like Receptors and Surfactant protein D are powerful modulators of the chemotaxis and primary immune response in *H. pylori* infection[108-110]. The infiltration of polymorphonuclear cells establishes the primary infection associated with acute gastritis[111]. As *H. pylori* antigens are presented to cells in the lymphatic organs, the humoral and cell-mediated immune responses are activated. Approximately 4 weeks after the initial infection, antibodies against *H. pylori* appear in the blood. The infiltration of mononuclear lymphocytes changes the inflammation from an acute inflammation to chronic superficial gastritis[111].

1.2.4 Clinical importance

Acute infection may be associated with a transient mild illness characterized by epigastric pain and nausea, but may also pass unnoticed. Most symptoms usually resolve within 2 weeks. Clinical data on acute infection is based mainly on a number of cases where investigators and volunteers have been infected[112]. Probably in the majority of patients, *H. pylori* does not cause symptoms, and the infection persists without any clinically evident disease[112].

In adulthood, *H. pylori* infection is involved in the pathogenesis of gastritis and peptic ulcers and is associated with gastric adenocarcinoma and MALT lymphoma[111]. *H. pyloris* role in gastrointestinal disease in childhood is yet incompletely understood. *H. pylori* is considered to be the major cause of the duodenal ulcer which is very uncommon in pediatrics, whereas the association between *H. pylori* and more common illnesses like non-ulcer dyspepsia, recurrent abdominal pain and gastric outlet obstruction remains controversial[101;113]. In recent years, a variety of extradigestive disorders, including migraine, cardiovascular diseases, autoimmune disorders, and liver diseases have also been related to *H. pylori* infection[114]. Marshall, the discoverer of the *Helicobacter* bacilli, postulated in 1997 a link with SIDS[115]. The hypothesis initiated a few case-control studies which did not produce consistent findings[116;117], and Marshall later rejected the hypothesis[118].

1.3 Surfactant and the collectins

1.3.1 Physiological properties of surfactant

Pulmonary surfactant is a complex of lipids and proteins that lines the interior of the lung. Presence of surfactant has been detected in the lungs of all major vertebrates and it has been argued that the evolution of the surfactant system must have been a prerequisite for the evolution of airbreathing[119]. Surfactant prevents alveolar collapse by reducing the surface tension across the air/liquid interface of the alveoli[120]. Lack of surfactant causes a disturbance of alveolar gas exchange. This can be seen in premature infants suffering from respiratory distress syndrome (RDS), a major cause of neonatal death. Introduction of surfactant replacement therapy has significantly reduced the morbidity and mortality of preterm infants. Surfactant is also involved in several defense mechanisms in the lung. It prevents the increase in airway resistance in allergen-challenged animals[121] and enhances mucociliary clearance of harmful contaminants[122].

The surfactant complex consists of approximately 90% lipid and 10% protein. Phosphatidylcholine is the dominant lipid and is present in disaturated form (dipalmitoylated phospatidylcholine DPPC) that enables surfactant to withstand very high surface pressure, thus preventing alveolar collapse. Other phospholipids and cholesterol constitute the major remaining lipids. Partial atelectasis of the lungs is a common feature at autopsy of SIDS victims, but is also found in other causes of infant deaths. Talbert and Southall proposed that a defect in surfactant composition or synthesis may trigger the death mechanism in SIDS[123]. They hypothesized that defective surfactant at a critical period in lung development may cause large areas of the lungs to collapse suddenly, greatly reducing the oxygen stores. However, the significance of the hypothesis remains unclear and surfactant has not received much attention from SIDS researchers in later years.

1.3.2 Surfactant proteins

Four surfactant proteins have been identified, SP-A, SP-B, SP-C and SP-D. These are produced and secreted into the conductive airways and alveolar space within the surfactant lipid-protein complex mainly by non-ciliated bronchial cells and pulmonary type II pneumocytes.

Much of the current knowledge of the surfactant proteins have evolved from studies on transgenic animals. SP-B and SP-C are small hydrophobic molecules integrated in the phospholipid layer and are involved in reducing the surface tension. Lack of SP-B causes a fatal respiratory failure in infancy, characterized by dysmorphic type II alveolar cells with small lamellar bodies, and alveolar collapse[124]. Lack of SP-C results in severe lung disease resembling interstitial pneumonitis and severe emphysema[124]. In humans, mutations in the SP-B gene inherited as an autosomal recessive genetic disorder have been identified, resulting in death in the neonatal period[125]. Distinct mutations in the SP-C gene have also been identified in humans and are associated with increased risk for respiratory distress, interstitial pneumonitis and the development of chronic lung disease[125].

SP-A and SP-D are large hydrophilic glycoproteins. Their main function is related to host defense and regulation of inflammation. SP-A is the most abundant surfactant protein and is believed to play a role in organizing the structure and affecting the function of surfactant lipids[126;127].

1.3.3 The collectins

The collectins consist of SP-A, SP-D and the serum protein mannose-binding lectin (MBL). They are powerful constituents of the innate immune defense, involved in opsonization, chemotaxis and clearance of pathogens[88]. They are characterized by a collagen-like triple helical region linked to a carbohydrate recognition domain (CRD) at the carboxy terminal end (Figure 5). SP-A is mainly produced in respiratory epithelium[128;129], whereas SP-D has been located in epithelial cells on all mucosal surfaces, and has been called the innate counterpart of IgA in the adaptive immune system[130]. MBL is an acute phase protein produced by hepatocytes. The collectins are large macromolecules that are joined in the N-terminal region and present in oligomeric forms. The tertiary structure of SP-D resembles a cart-wheel with a diameter of more than 100 nm and with the CRDs radiating out to the perimeter[131] (Figure 5). The CRDs of the collectins are able to bind calcium-dependently to lipid and carbohydrate-derived microbial substances. Bacterial cell wall components like lipoteichoic acid of Gram-positive bacteria and lipopolysaccharides (LPS) of Gram-negative bacteria are ligands for the collectins. Most of these components occur as

repeating units that enhance the interaction between collectins and microbes. Normal mammalian cells do not present such pathogen-associated molecular patterns characteristic of microorganisms.

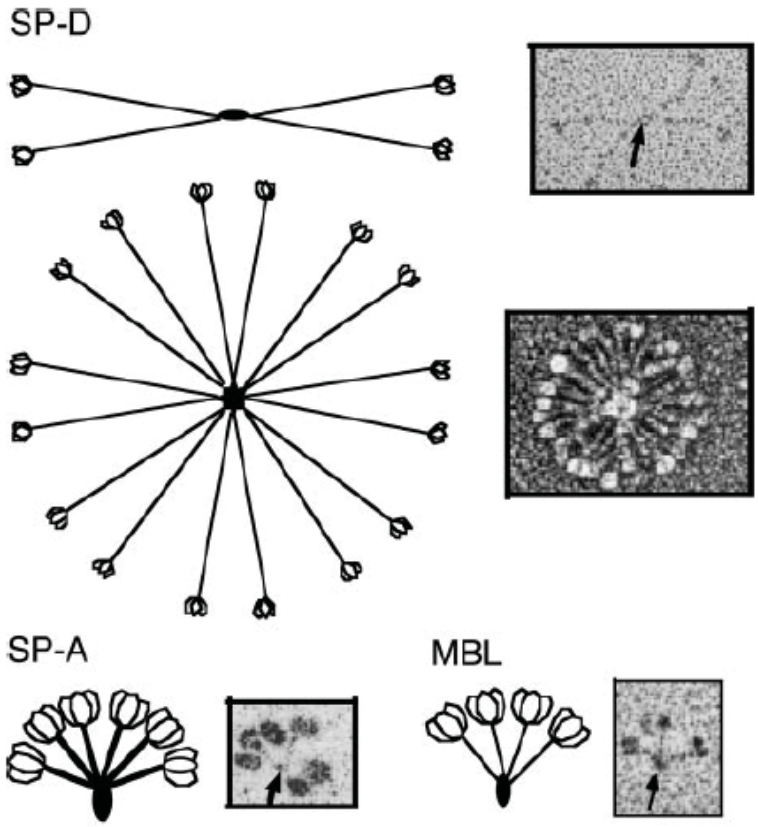

Figure 5. Schmatic drawing and electron microscopic photographs of collectins. From Holmskov et al. 2003[88].

MBL has the ability to activate complement through an independent pathway. Clinical studies have disclosed that MBL insufficiency increases morbidity of respiratory tract infections in early childhood[87] and is critical in avoiding sepsis and septic shock in critically ill patients[132]. The binding of SP-A and SP-D to microbial surfaces leads to inhibition of bacterial and fungal growth[133;134], opsonization for phagocytosis by macrophages[135] as well as aggregation and neutralization[136]. SP-D has been shown to play a role in the host response to *H. pylori* infection, being able to agglutinate and greatly reduce the motility of *H. pylori* bacteria[108]. SP-A and SP-D transgenic mice subjected to bacterial infection present compensatory cytokine expression, increased inflammation and oxidant production[137].

2 Objectives of the study

1. To investigate the prevalence of *Helicobacter pylori* infection in Norwegian newborn infants and small children. Furthermore to detect possible epidemiological associations of *H. pylori* infection with factors such as mode of delivery, nutrition, household members and parents' country of origin. Previous studies from western countries have detected low prevalence of *H. pylori* infection in small children, but few have investigated very young infants and neonates. Developmental studies are required in SIDS-research for comparative investigation. (Paper I)

2. To study the role of *Helicobacter pylori* infection in SIDS and other sudden unexpected deaths in infancy. A link between *H. pylori* and SIDS was proposed in 1997. Small, qualitative studies have thus far failed to provide evidence of a significant association, but large sample investigations have not been performed.

3. To investigate the developmental features of the innate component Surfactant protein A (SP-A) by evaluating the immunohistochemical expression in human lung tissue. The aim was to develop a method for evaluation of SP-A immune staining in order to investigate a possible role for SP-A in SIDS.

4. Some genetic variants of mannose-binding lectin, MBL, are associated with low plasma levels and a clinical state of minor immune deficiency. The first goal of this study was to investigate whether the two other collectins: surfactant protein A (SP-A) and surfactant protein D (SP-D) also may be genetically determined. The association between genetic variants and the immunohistochemical expression of SP-A and SP-D has not been studied previously. The second goal of this study was to investigate possible associations between SP-A, SP-D and MBL genotypes and SIDS.

3 Material and Methods

3.1 Subjects and tissue samples

The patients were cases of sudden unexpected deaths in infancy investigated at the Institute of forensic medicine, University of Oslo in the period between 1989 and 2004. Included in paper III are a few cases of intrauterine fetal deaths as well as cases of sudden unexpected deaths in childhood and in adulthood. The median time-interval from death to autopsy was 19 hours (range 5-77) and all autopsies were performed by the same forensic pathologists (TOR, ÅV, MAR, ASP), according to the Nordic diagnostic criteria for SIDS[39]. Clinicopathological information about the patients is given in the respective papers (II, III, IV). In papers II and IV only infants of ethnic Norwegian citizen were included in the studies, to avoid selection bias due to the high prevalence of *H. pylori* among immigrants[102] and to take advantage of the genetic homogeneity of the Norwegian population. Body fluids and tissue samples were obtained during autopsy.

The Nordic SIDS criteria[39;41] implies that no cause of death has been revealed after a thorough evaluation of the circumstances of death, review of the medical history and a full post-mortem investigation including total body skeletal X-ray, toxicological and microbiological investigation. Examination of the death scene was performed by forensic experts between 2001 and 2004. In the cases from 1989-2000, medical doctors/health personnel and/or the local police had been called to the scene according to Norwegian regulations.

Included in papers I and II were also live infant controls, who were recruited in either the maternity ward at Rikshospitalet in Oslo or in outpatient mother-and-child clinics in the cities of Drammen and Droebak (Marienlyst and Frogn helsestasjon). From the newborn infants, the fecal (meconium) specimens were taken right after their first defecation at day 1 or 2 after birth. In the outpatient clinics, infants and children who presented with a fresh sample of stool in their diapers were included. A few parents also brought to the outpatient clinic a fresh sample of the child's stool collected at home.

3.2 HpSA ELISA

Stool specimens were stored in an ultrafreezer at -80^{0}C. The Premier Platinum *Helicobacter pylori* Stool Antigen (HpSA) test (Meridian Bioscience Inc., Ohio, USA) was performed according to the instructions of the manufacturer. Approximately 200 mg of feces was diluted and incubated in microwells coated with polyclonal rabbit antibodies recognizing *H. pylori* antigens and then with rabbit anti-*H. pylori* antibody conjugate in the presence of color developing solution. The OD values were determined at 450 nm wave length. According to the manufacturer, tests were to be regarded positive when OD $\geq$ 0.160, equivocal when 0.140 $\leq$ OD $\leq$ 0.160, and negative when OD $\leq$ 0.140. All samples were tested twice using dissimilar test tubes and reagents, and performed blind without knowledge of the other test results. Both tests had to yield an OD $\geq$ 0.160 for a positive result.

3.3 DNA extraction and PCR

3.3.1 *H. pylori*

To extract bacterial DNA from feces, the QIAamp Stool Mini Kit (Qiagen GmbH, Hilden Germany), was used. The method is supposed to allow removal of DNA-damaging substances and PCR inhibitors present in the stool. The DNA extraction was performed according to the manufacturer's instructions with minor modifications suggested by Sicinschi et al[138]. A *H. pylori* strain from the local collection at the Institute of Microbiology, Rikshospitalet, was used as positive control for the PCR assay. DNA from *H. pylori* was isolated using Prepman Ultra (Applied Biosystems, California, USA).

PCR amplification with *Helicobacter* specific primers was performed in 25-µl reaction volumes containing Tris buffer. The primers consisted of two specific 16S rDNA oligonucleotides, which generated a 138-bp DNA product[139]. Fifty thermocycles were performed at 60^{o}C and the DNA lysates were analyzed by electrophoresis on an agarose gel, each trial included a positive and a negative control.

PCR products and primers were sent via http://www.medprobe.com to Lark Technologies (Essex, UK) who performed sequencing. Obtained sequences were analyzed with the Sequence Scanner 1.0 program (freeware) and subjected to homology search analysis using the NCBI databases and BLAST algorithm http://www.ncbi.nlm.nih.gov/BLAST/.

3.3.2 Collectins

DNA was extracted from blood/spleen using standard extraction methods. In patients in whom specimens of blood or spleen were not available, DNA was extracted from alcohol-fixed paraffin-embedded tissue by removal of paraffin and digesting with proteinase (paper IV).

A sequence specific primer-PCR method as described by Pantelidis et al[140] was applied for genotyping of the single nucleotide polymorphisms (SNPs) in SP-A1 at aa19, aa50, aa62, aa133, and aa219, in SP-A2 at aa9, aa91, aa140 and aa223, and in SP-D at aa11 and aa160. To achieve reproducible results for the SNP at aa140 within SP-A2, the control primers were changed from the recommended 63/64 yielding a 784 bp control product to the one used for several of the other PCR reactions, 210/211, yielding a 249 bp product. Minor modifications in the amount of primers in the PCR reactions were also performed. The two SP-A genes have been shown to be in marked linkage disequilibrium. The SP-A1 and SP-A2 alleles cosegregate as one unit (haplotype) denoted 6An /1An[141]. The haplotype frequencies were determined on the basis of homozygous genotypes, and haplotyping from the heterozygous genotypes was based on the highest likelihood as described by Rämet et al[142].

The MBL genotyping was performed by the use of PCR and restriction fragment analysis. The primers, reaction conditions and restriction enzymes are given in paper IV. The different fragments were visualised under ultraviolet light by gel electrophoresis on agarose gels.

3.4 Immunohistochemistry

3.4.1 Material

Tissue samples of central and peripheral part of the lung, palatine tonsils, submandibular gland and the wall of the stomach and the duodenum were obtained at autopsy performed within 71 hrs of death (median 23 hrs). The sections were cut at 4 μm and fixed in 96% cold ethanol or 4% formaldehyde. Tissue samples showing signs of extensive autolysis were excluded from the study. A small selection of lung samples was fixed in 4% formaldehyde and processed for double immunohistochemical staining.

3.4.2 Staining procedures

The antibodies used in the immune staining studies with peroxidase (enzyme) technique were monoclonal mouse anti-human SP-A IgG_1(Hyb 238-4), mouse anti-human SP-D IgG_1 (Hyb245-1), mouse anti-human CD 68 IgG_3 (Clone PG-M1, DAKO, Glostrup, Denmark), and mouse anti- *H. pylori* IgG (B-0471, DAKO) (Table 2). Immune staining was performed manually (SP-A, SP-D) or automated (*H. pylori*) with the ChemMate EnVision Detection Kit Peroxidase/DAB+ (K5007; DAKO), which includes (I) applying a peroxidase blocking solution to avoid unspecific staining, (II) incubating sections with the target antibodies, (III) incubating with polymer which adheres to the target antibodies and (IV) applying substrate to yield specific DAB+ (horseradish brown) staining of targets. Counterstaining was finally briefly performed with hematoxylin (purple blue).

Double staining was performed in a small selection of lung tissue specimens with the ChemMate EnVision G/2 Doublestain System (K5361; DAKO), targeting SP-A and additionally CD 68 for identification of phagocytes. Following incubation with primary antibody SP-A, peroxidase polymer and DAB+ (horseradish) substrate, the slides were washed and specimens covered with Doublestain Blocking solution, and subsequently incubated with CD 68 antibody, alkaline phospatase polymer and Permanent Red substrate.

Table 2. Specifics of the antibodies used in immunohistochemical staining

Specificity	Product	Ab-type	Working dilution	Stock concentration	Peroxidase substrate	Fluorescence conjugate
SP-D	Mouse anti-human	IgG_1	1:1200	1 mg/ml	DAB	
SP-A	Mouse anti-human	IgG_1	1:5500	1 mg/ml	DAB	
CD 68	Mouse anti-human	IgG_3	1:200	1 mg/ml	Permanent red	
H. pylori	Rabbit anti-human	IgG	1:40	0.32 mg/ml	DAB	
IgA	Goat anti-human	IgG	1:40	1 mg/ml		FITC
IgM	Goat anti-human	IgG	1:20	1 mg/ml		TXRD
IgG	Goat anti-human	IgG	1:20	1 mg/ml		TXRD

The expression of IgA, IgG and IgM immunocytes in the gastric and duodenal mucosa was evaluated by paired immune fluorescence technique as described in previous studies[80;82]. Briefly, alcohol-fixed 4 μm thick sections were incubated with fluorochrome-conjugated goat anti-human antisera to the immunoglobuline isotypes IgA, IgM and IgG (Southern Biotechnology Associates, Birmingham, Alabama, USA). Antibody reagents were applied in pairs of contrasting colors (FITC – fluorescein isothiocyanate (green) or TXRD – texas red).

3.4.3 Microscope and photography

Observations were performed using low (40x - 400x) power light microscope. For examination of *H. pylori* presence in gastric tissue, high power (630x) light microcopy was performed. A Leitz Aristoplan fluorescence microscope equipped with a Ploem-type epiilumminator for narrow band excitation and selective filtration of green and red emission colors was used for evaluation of immune fluorescence staining.
Digital photographs were captured using a Leitz digital camera and the softwares Leica/Adobe photoshop 2.0.

3.4.4 Evaluation of immune staining

Based on the observed staining pattern, a semi-quantitative scoring system was developed for evaluation of the intensity and distribution of SP-A and SP-D immune staining in lung tissue. A scale from 0 to 3 was applied: With regard to intensity, a score of 0 represents no evident staining, 1 represents weak staining, 2 – moderately strong staining and
3 - strong staining. With regard to distribution, a score of 0 means virtually no staining of cells, 1 represents less than 25% cells stained, 2 – between 25-50% of cells stained (25-75% for SP-A) and 3 – staining of more than 50% of the cells (75% for SP-A). A total score for SP-A and SP-D expression in the lungs as well as for SP-D expression in submandibular gland was calculated by multiplying the distribution and intensity scores. The detailed characteristics of the scoring systems are given in paper III and IV.

With regard to the density of immunoglobuline producing cells, counting of immunocytes in the gastric/duodenal mucosa was performed by an ocular grid with grid area 0.01 mm^2. Presence of IgA, IgM and IgG plasma cells (immunocytes) in the lamina propria and submucosa were counted, as reported by Stoltenberg et al[80]. The density of immunocytes in each sample was based on counting 20-25 grid fields which has been shown to be necessary to obtain a stable mean[80;82]. Only areas with preserved histologic structures were examined. These results are shown in figure 7 in the discussion below, but are not presented in any of the papers.

The *H. pylori* immune staining in gastric tissue was evaluated blindly by a specialist in microbiology and pathology (ÅV) who had no information about case history or stool test findings.

3.4.5 Reproducibility

Blind evaluation of SP-A staining pattern and staining intensity by two different observers (ASP and TOR) showed a high degree of agreement (SP-A: Kappa=0.80). A blinded trial performed by the same observer on two different occasions showed a high degree of scoring reproducibility (SP-A: Kappa=0.90).
Also for counting of immunocytes inter- and intraobserver reproducibility was satisfactory (Kappa=0.74 and 0.60 respectively). The *H. pylori* immune stains were reevaluated blindly by ÅV and the reproducibility was good having the same result in 24 out of 26 reexamined cases.

3.5 Interleukin-6 measurement

Interleukin-6 (IL-6) concentrations in CSF were measured by an ELISA kit (R & D Systems Inc., Minneapolis, USA), utilizing 100 µl CSF and determining Optical Density at 450 nm as previously described[90].

3.6 Statistical analysis

The two-tailed Mann-Whitney U test was used for comparison of non-parametric variables between two groups. The frequencies of categorical variables such as individual alleles and haplotypes between the SIDS and control groups (paper IV) were compared using 2 x 2 tables and the use of the χ^2 test. When the expected cell values were <5, the Fisher's exact test was applied. Bivariate logistic regression was used to model the associations between *H. pylori* status and clinical variables (paper I), and to compare high/low SP-A expression scores and clinical variables (paper III). For testing variation in total SP-A scores with age, the Kruskal–Wallis one-way analysis was performed (paper III). For testing of inter- and intra-observer reproducibility, the Kappa test was applied (paper III and IV). The level of significance was set to 0.05. Analyses were implemented by the use of SPSS version 14.02 (SPSS, Chicago IL, USA).

3.7 Registry and approval

All studies were approved by the Committee for Medical Research Ethics in Southern Norway (REK sør, ref. nr. S-02272). The *Helicobacter* studies (paper I and II) were presented and approved by the Norwegian Social Science Data Services Detection (NSD, ref.nr 11414). The usage of autopsy material from a registered Biobank obtained during forensic investigations of infants for research purposes, has been approved by the Norwegian Dept of Health. The fecal specimens collected from live healthy infants also constitute a registered Biobank.

4 Summary of the results

In this chapter the results from each paper are summarized.

4.1 Publication I

Stray-Pedersen A, Gaustad P, Stray-Pedersen B and Rognum TO. ***Helicobacter pylori*** **stool antigen detection rate in newborn infants and small children.** J Perinat Med. 2007;35(2):155-8.

Gastrointestinal colonization with *Helicobacter pylori* is generally believed to occur in infancy and result in a chronic lifelong infection. The aim of this study was to investigate the prevalence of *H. pylori* infection in normal healthy neonates, infants and small children in Norway.

Fecal samples from 249 Norwegian children aged 0 days -3 years were collected and tested for the presence of *H. pylori* antigen using the Premier Platinum HpSA immunoassay. For verification purposes, 52 samples (26 HpSA positive and 26 negative) were were analyzed with PCR targeting the 16 S rDNA *Helicobacter* gene and the PCR products were sequenced.

Results: *H. pylori* antigen was detected in the feces from 52% (36/69) of the newborn infants, 15% (7/46) of infants aged between 7 days and 1 month, and 5% (7/134) of children aged between 1 month and 3 years. The *H. pylori* antigen detection rate in newborn infants was significantly associated with birth manner; 59% (30/51) of infants with normal vaginal births tested positive compared to only 10% (1/10) of infants delivered by cesarean section (p=0.02). Positive PCR results were found in 35% (9/26) of HpSA positive and 12% (3/26) of HpSA negative samples. Sequencing of PCR products revealed 97-100% homology with gene sequences from both *H. pylori* and other *Helicobacter* species.

Conclusions: The low *H. pylori* antigen detection rate in small children older than one month of age is in accordance with previous studies from western countries. The striking finding of a high *H. pylori* antigen detection rate in newborn infants, suggests that transient *H. pylori* colonization may occur in the neonatal period and that transmission of *H. pylori* may be related to birth and mode of delivery.

4.2 Publication II

Stray-Pedersen A, Vege Å, Musse M, Rognum TO. ***Helicobacter pylori*** **antigen in stool is associated with SIDS and sudden infant deaths due to infectious disease.** Pediatr Res. 2008 Jun 4. [Epub ahead of print]

A possible link between *Helicobacter pylori* infection and SIDS was hypothesized in 1997, but has gained limited support by later studies. To test the hypothesis, stool specimens from a retrospective cohort of 160 sudden unexpected deaths in infancy and 156 live controls were investigated.

The patients included in the study were 122 SIDS victims, 17 deaths due to infection, 10 due to non-infectious disease and 11 accidental/violent deaths. The stool specimens were obtained at autopsy and stored at -80^{o}C. Stool from live control infants were obtained from their diapers. Presence of *H. pylori* antigen was detected using the HpSA immunoassay. IL-6 concentrations in CSF were measured in 147/160 cases. Gastric tissue specimens were evaluated by Giemsa and immunoperoxidase staining in 12 HpSA positive and 14 negative SIDS cases.

Results: *H. pylori* antigen was detected in 8% (12/156) of the live controls compared to 25% (30/122) of SIDS cases ($p<0.001$), 53% (9/17) of deaths due to infection ($p<0.001$), 20% (2/10) of deaths due to non-infectious disease ($p=0.20$), and 9% (1/11) of accidental/violent deaths ($p=0.60$). In the classic age peak for SIDS, 1-5 months, 31% (21/67) of SIDS cases were HpSA positive compared to 1.5% (1/68) of live controls ($p<0.001$). Rod-like immunoperoxidase positive *H. pylori* organisms were identified in 7/12 HpSA positive gastric antrum sections compared to 2/14 HpSA negative ($p=0.038$). Significantly elevated IL-6 levels in CSF were demonstrated in HpSA positive SIDS victims compared to HpSA negative victims ($p=0.045$).

Conclusion: Detection of *H. pylori* antigen in stool is associated with SIDS and deaths due to infections. We hypothesize that *H. pylori* infection in infancy may be involved as the triggering pathogen for sudden death during the first five months after birth.

4.3 Publication III

Stray-Pedersen A, Vege A, Stray-Pedersen A, Holmskov U, Rognum TO. **Post-neonatal drop in alveolar SP-A expression – biological significance for increased vulnerability to SIDS?** Pediatr Pulmonol. 2008 Feb;43(2):160-8.

Surfactant protein A (SP-A) is a water-soluble large glycoprotein secreted into the lung alveoli by bronchiolar epithelial cells and type II pneumocytes. SP-A has a structural role within the surfactant system to prevent alveolar collapse, and being a part of the of the innate immune system, SP-A is a powerful modulator of inflammatory processes. The aim of this study was to evaluate the expression of SP-A in human lung tissue at various ages from fetal life to adulthood, with special regard to possible implications for sudden infant death syndrome (SIDS). Lung tissue specimens from 13 intrauterine-, 6 neonatal-, 108 infant-, 19 childhood- and 14 adult deaths were obtained at autopsy. Fifty-nine of the infant deaths studied were classified as SIDS, 29 had succumbed due to a disease and 20 were accidental/violent deaths. Immunohistochemical detection of SP-A using monoclonal antibodies was performed by microscopy of lung tissue specimens collected at autopsy. A scoring system was developed enabling semi-quantitative estimation of staining intensity and distribution.

Results: SP-A was detected in the terminal bronchioli and alveolar spaces of fetuses >35 weeks gestation. The intra-alveolar SP-A expression increased in the perinatal period followed by a marked drop in infants aged between 1 week and 5 months. Infants older than 5 months of age had abundant SP-A expression corresponding to older children and adults. There was no difference in the age distribution between cases of SIDS and explained deaths.

Conclusions: The apparent drop in SP-A expression takes place in the first months after birth, corresponding with the classical age peak of SIDS. We therefore hypothesize that low expression of SP-A may be related in some as yet undetermined way to the increased risk of SIDS at that age.

4.4 Publication IV

Stray-Pedersen A, Opdal SH, Vege A, Moberg S, Rognum TO. **Surfactant protein A and D genetic variants and protein expression – a possible association with Sudden infant death syndrome.** Submitted*.

Gene polymorphisms in surfactant protein A (SP-A) and surfactant protein D (SP-D) has been associated with several respiratory disorders in infancy and genetic variants of mannose-binding lectin (MBL) are known to increase the susceptibility to infections. We performed genotyping of SP-A, SP-D and MBL in infants that had died suddenly, and investigated whether immunohistochemical expression of SP-A and SP-D is dependent upon the variants of the genes examined. Genotyping of the SP-A genes (SP-A1 and SP-A2), SP-D and MBL was performed in 42 SIDS and 46 explained sudden infant deaths. Immunohistochemical SP-A and SP-D expression in tissues from lung and upper alimentary tract was evaluated by a semi-quantitative scoring system.

Results: Immunohistochemical SP-A and SP-D expression in tissues from lung and upper alimentary tract was evaluated by a semi-quantitative scoring system. The most common SP-A haplotype, 6A2/1A0, tended to be overrepresented in the cases with low immunohistochemical SP-A expression (61%) compared to cases with high expression (49%), p=0.08. The SP-D expression was not determined by the 11 C/T or 160 A/G single nucleotide polymorphisms. Homozygous allele variants of MBL which are associated with low plasma levels, were found in only 1% of sudden infant deaths.

Conclusions: Though no significant relationships between the investigated SP-A and SP-D gene variants and the SP-A and SP-D tissue expression were found, the significance of the SP-A haplotype 6A2/1A0 should be further explored. No association between SIDS and the genetic variants of SP-A, SP-D and MBL is indicated by the present study.

* Revised manuscript accepted for publication in Acta Paediatr.

5 General discussion

The four original papers, on which this thesis is based, represent studies regarding the role of *H. pylori* infection and the innate immunity components SP-A, SP-D and MBL in infancy with special emphasis to SIDS. In this section, the study findings will be discussed in the context of the Fatal triangle explaining SIDS (Figure 2).

5.1 The Fatal triangle

The fatal triangle for SIDS[38] was inspired by Valdes-Dapena's suggestion from 1985[143] that SIDS might be a bi-phasic phenomenon:

1. Before birth the infant may be subjected to adverse intrauterine influences, e.g. maternal smoking, poor maternal nutrition and maternal anaemia, making it subtly functionally handicapped and thus predisposed to SIDS after birth.

2. After birth, the infant may be "challenged" by a trigger - some external or internal factor with which he or she could not cope, e.g. an upper respiratory infection, an episode of regurgitation and choking, etc.

Bearing in mind the typical peak of SIDS occurrence between the second and the fourth month after birth especially significant during the 1980'ties, Rognum and Saugstad added a third phenomenon: a vulnerable developmental stage and included *genetic make-up* as an additional predisposing factor[38]. It soon turned out that other researchers had similar ideas: Hannah Kinney's group at Harvard Medical School launched their triple risk model[144] and Kahn's research group proposed a multifactorial model including three sets of factors[145].

In 2002 Guntheroth and Spiers[36] discussed the different models and stated that in fact Wedgewood already in 1972 had grouped risk factors into the first "triple risk hypothesis". The risk factors consisted of general vulnerability, age specific risks and precipitating factors. Guntheroth and Spiers stated that all three triple risk hypotheses are fairly similar[36]. The fatal triangle is shown above (Figure 2).

5.1.1 Predisposing factors

Predisposing factors may origin from fetal life and be due to a variety of adverse intrauterine influences, e.g. maternal smoking, maternal nutrition, maternal anemia[143]. One of these factors, brain stem astrogliosis, may have been caused by hypoxic events[22,27], and also theoretically by infection[82;146]. Brain stem astrogliosis is described in SIDS by several authors[23-25;147] .

The genetic "make-up" i.e. individual variants in the genome may also constitute predisposing factors. Several studies have aimed at finding the SIDS gene[148], however only a few of the proposed candidates genes have thus far been supported by evidence:

- Substitutions, mutations and genetic variants of mitochondrial DNA have been reported in individual cases but no predominant mtDNA mutation has so far been found to be associated with SIDS in case control studies[163].

- Polymorphisms in the IL-10 gene have been found to be associated with sudden deaths in infancy due to infectious disease and is perhaps also playing a role in a proportion of SIDS cases[93; 149; 150].

- Another investigated candidate has been the IL-6 gene: A genotype determined by a single nucleotide polymorphism (174 G/C) has been found in increased frequency in Australian[91] and British SIDS victims[151], but not in Hungarian or German[91], and not in Norwegian SIDS victims[92].

- Partial deletions of the complement factor 4 (C4) gene in combination with slight upper airway infections have been related to an increased risk for SIDS[152-154].

- Genes involved with thermal balance has so far not been related to SIDS[155;156].

- Recent studies on genetic variants in the Serotonin transporter (5-HTT) gene also seem compelling. An increased frequency of a long allele polymorphism, giving an especially high serotonin reuptake, has been found in Japanese SIDS victims[157], in black[158], and white American victims (Paterson et al - abstract presented at International congress on sudden unexpected death in Infancy, Soria Moria, Oslo, Nov 2007), and in Norwegian victims (Opdal et al 2008: personal communication). An insertion mutation possibly affecting 5-HT neuronal development was also recently reported in 6 out of 49 black American SIDS victims compared to none of the controls[159].

- Genetic variants of the Long QT syndrome (LQTS) genes were recently demonstrated in 19 of 201 cases originally diagnosed by us as SIDS[17]. The genetic alterations were shown to be functionally significant by electro-physiologic analysis, implying that the cause of death was cardiac arrhythmia rather than SIDS in 16 of the cases, whereas in 3 cases the gene variant may have served as a predisposing factor for SIDS, exhibiting depolarization shift under conditions of internal acidosis[161]. Furthermore, a newly discovered mutation (GPD1-L) associated with alteration in the function of cardiac sodium channels was recently reported in 3 out of 304 German infants, originally classified as SIDS[162].

5.1.2 Vulnerable developmental stage

The “critical” age peak for SIDS has been shown to be from 2 to 4 (5) months of age in different populations[10;50;52;160]. These first post-natal months represent a critical period for homeostatic control. The infant goes through a period of “reflex chaos” in which fetal reflexes vanish and aimed voluntary movements are not yet developed[13]. The consequence may be the inability to turn the head to the side in case of a dangerous prone sleeping position with the face down in a soft mattress.

Other developmental changes in autonomic nervous control, i.e. changes in respiration and heart rate, also occur at this stage of development[164]. Wailoo’s research group has shown that infants change from infant- to adult-like night-time body temperatures at some individual point between 2 and 4 months of age[71]. Changes in night-time heart rate and deep sleep duration occur at the same time[71].

The immunologically "naive" newborn infant is protected against infections by maternally-derived antibodies. After the perinatal period, in the first months of life, these antibodies disappear and following the exposure to various microbes, the infant's ability to generate a primary immune response is generated[84-86].

5.1.3 Trigger event

The vast majority of infants of mothers who smoke and infants carrying genetic predisposition for immunological dysfunction, survive the vulnerable phase. The trigger initiating the death mechanism may be bed-sharing in inappropriate circumstances, or the combination of prone sleeping and a slight infection. A relationship between SIDS deaths and the month-to-month variation in *Bordetella pertussis* infection has been shown, and in Norway during a whooping-cough epidemic, a significant increase in SIDS rates was observed[74]. Respiratory virus RNA has been detected by PCR in lung tissue more frequently in SIDS victims than in non-SIDS cases[77]. The seasonal variation involving a winter peak is considered typical not only for SIDS, but is also an observable phenomenon in respiratory virus epidemics, and it has been hypothesized that the SIDS rate is affected by outbreaks of respiratory virus epidemics[75;76].

We have compared the SIDS rate in Norway with the registered numbers of Respiratory Syncytial Virus (RSV) and Influenzae virus identifications reported to the Norwegian Institute of Public Health (abstract presented at International congress on SUDI, Soria Moria, Oslo, October 2004). Although the dominant feature was that the SIDS rate decreased from 37 cases in 1993 to 20 cases in 2005 (Figure 6), minor changes in the SIDS rate seem to follow the biannual peak of virus epidemics.

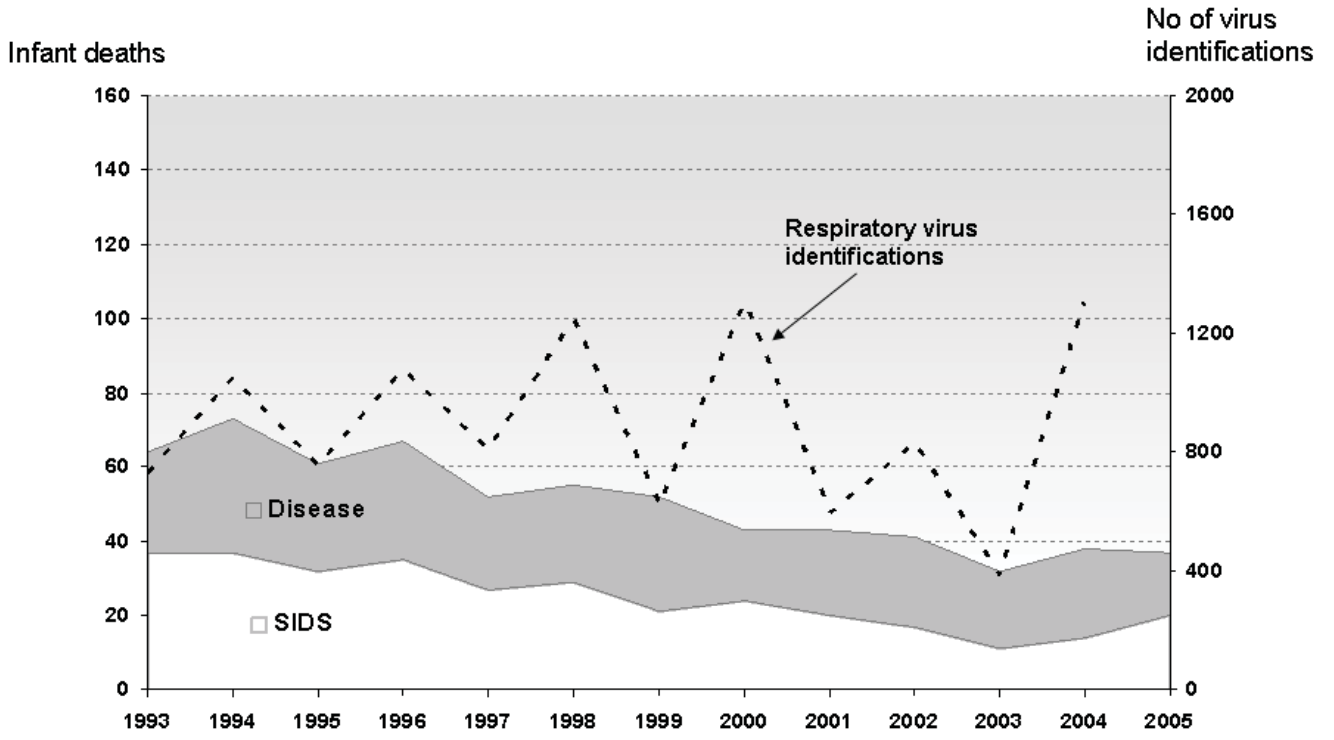

Figure 6. **Comparison of infant mortality rate and respiratory virus identifications.** Areas represent the post-neonatal infant mortality rates due to SIDS (white) and disease (infections, tumors, neurological disorders) (grey). Stapled line represents the number of patients with positive identifications of Respiratory Syncytial Virus (RSV) and Influenza virus in the peak month of the winter epidemics. Virus identifications show a biannual pattern. Although the dominant feature was that the SIDS rate decreased from 37 cases in 1993 to 20 cases in 2005, there seems to be a slight increase in incidence of SIDS in years with virus identification "tops". Datasets derived from Statistics Norway and Norwegian Institute of Public Health (Courtesy of Dr. G. Ånestad).

5.2 Helicobacter pylori – a possible role in SIDS?

5.2.1 Marshall's hypothesis

In 1997, Pattison and Marshall proposed that *H. pylori* infection[115] may play the role of an exogenous trigger in SIDS. They hypothesized that *H. pylori* infection may result in production of large amounts of urease which, if aspirated with gastric juice, could reach the alveolae, react with plasma urea, and produce ammonia toxicity inducing respiratory arrest. An alternative postulated mechanism may be release of inflammatory cytokines induced by *H. pylori* infection, resulting in disturbed homeostasis of the immune system, inducing high fever and increased deep sleep leading to critical hypoxemia, coma and death[115].

5.2.2 Epidemiological evidence

The association between *H. pylori* infection and SIDS is to some extent supported by epidemiological data. Both *H. pylori* infection and SIDS is associated with poor socioeconomic conditions[49;165]. SIDS is a disease of infants younger than one year[10]. In developing countries, it has long been appreciated that *H. pylori* infection occurs within the first year of life[100]. In paper I, evidence is brought to support that infants in a developed country also encounter *H. pylori* in early infancy. It has been argued that the SIDS rate in developing countries is low[166], but this is most likely due to poor post mortem investigations and over-shadowing by a huge infant mortality rate due to infectious disease. However, other risk factors for SIDS such as male gender predominance, prematurity and maternal smoking, have thus far not shown any association with *H. pylori* infection.

5.2.3 Previous experimental work

In the year 2000, Kerr et al reported PCR findings suggestive of *H. pylori* infection in 28 of 32 SIDS cases compared to only 1 of 8 controls and concluded that a causative role for *H. pylori* in SIDS was likely[116]. The study was heavily criticized, the study findings were argued to result of false positive PCR products due to contamination[167]. Four subsequently published small-number studies were unable to confirm Kerr's results[117;118;168;169]. *H. pylori* bacilli were not documented histopathologically in any of the studies.

5.3 Methodological considerations

We selected a different approach to investigate the role of *H. pylori* in SIDS, designing a large sample case-control study and using the *H. pylori* stool antigen (HpSA) test to detect *H. pylori* infection - a test which has been developed particularly for pediatric populations[170]. In paper II we present that detection of *H. pylori* antigen is significantly associated with SIDS compared to live controls (Paper II). The validity of the test is confirmed by histology. The methodological and clinical considerations concerned with the study findings are discussed below.

5.3.1 The accuracy of the HpSA test

The HpSA ELISA test has been widely used in clinical pediatric practice with an adequate sensitivity and specificity for detecting *H. pylori* infection, between 85-100% and 82-99% respectively, which is comparable to the Urea breath test[171]. The diagnostic accuracy has been decent in both high and low prevalence populations[170;172]. The test utilizes polyclonal antibodies, and has now been replaced by a monoclonal kit (HpSAplus) with only slightly improved precision[173]. However, the possibility of cross-reaction with other bacteria or antigens can not be ruled out with the polyclonal test.

5.3.2 Effects of decomposition and autolysis

Investigations on material obtained at autopsy are invariably influenced by autolysis. Decomposition of organ tissue begins shortly after death. Good controls are therefore a prerequisite for such research. We first performed the HpSA test in fresh stool samples from 249 infants and children. The HpSA test results in stool samples obtained during autopsy of victims of accidental deaths did not differ significantly from the age-matched live controls (9% and 8% respectively). Thus, the HpSA test seems to behave similarly in stool samples from live and dead infants. The interval between time of death and autopsy was less than three days in all cases, median 19 hrs.

5.3.3 *H. pylori* and the immune response

Curved or rod-like *H. pylori* structures were detected by histology, but signs of acute inflammation was not observed in any of the cases. The expression of IgA, IgG, and IgM immunocytes in the gastric mucosa was studied in 45 of the sudden infant deaths presented in paper II. However, this immune staining evaluation was not included in the final manuscript to avoid excess information. The amounts of IgA expressing immunocytes present in the gastric mucosa increased significantly with age the first 6 months of life. On the other hand, the amounts of IgG and IgM immunocytes were in most cases low for all ages. Similar observations have been made in other tissues previously, e.g. trachea and duodenum[85;86] . There was a trend that HpSA positive infants between 1 and 5 months of age showed a higher expression of IgA immunocytes in the gastric mucosa than HpSA negative infants. No differences were observed with regard to IgG and IgM expression (Figure 7). We speculate that the inability to detect significant mucosal immune stimulation and signs of inflammation, is largely due to autolysis. However, central immune stimulation, reflected by elevated IL-6 levels in CSF, showed a significant association with *H. pylori* infection.

5.3.4 *H. pylori* detection rate affected by age

Unanticipated findings are one of the thrills involved with basic medical research. Originally, we did not intend to investigate the *H. pylori* detection rate in live newborn control infants. The unexpected finding that the rate of *H. pylori* detection was higher in infants between 7 days and 1 month of age (15%) compared to all other age groups (3-10%) prompted the collection of samples from neonates. *H. pylori* antigen was detected in the meconium samples from more than half of the newborn infants (paper I). The OD-values of the positive tests were mostly well above the threshold of 0.160, with several of the tests above 2.0, similar to the positive control reagent supplied with the kit.

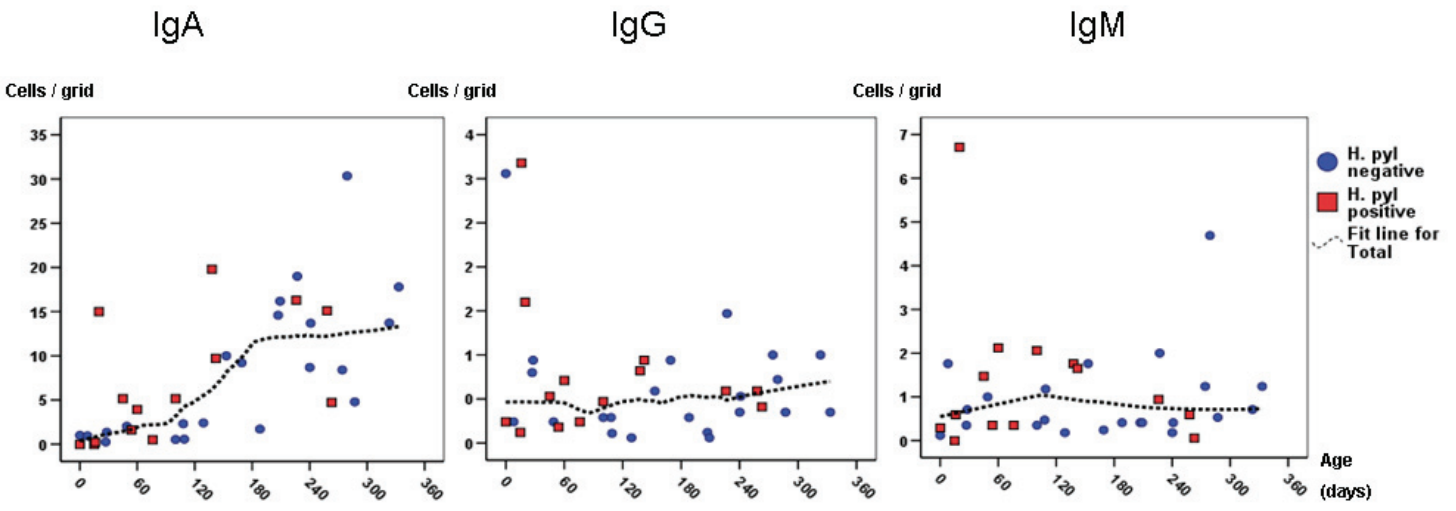

Figure 7. Epithelial immunofluorescence scores for IgA, IgG and IgM in the gastric mucosa. Red squares represent cases of infant deaths with positive *H. pylori* stool tests and blue circles cases testing *H. pylori* stool negative. Dotted line represent average (normal) fit line. The presence of IgA immunocytes is low in the first 120 days of life and increase rapidly at age 150-240 days. The number of IgA immunocytes present before 150 days, tends to to be higher in *H. pylori* positive infants than in *H. pylori* negative. Scattered IgG and IgM immunocytes are present throughout the first year of life, with no difference observed in *H. pylori* positive vs. *H. pylori* negative infants.

Fujimura et al has investigated stool samples from 50 Japanese infants at 3 days of age and found only one infant (2%) testing positive by the HpSA method, but 15 (30%) testing positive by PCR[174]. We are unable to clarify the discrepancy between their findings and ours. One difference between their study and ours is that we collected most of the samples within 12 hours after birth, and all within 2 days, whereas Fujimora et al obtained samples at day 3.

5.3.5 PCR of *H. pylori* DNA

In an attempt to confirm our results, we performed PCR analyses and verified the presence of *H. pylori* DNA in 35% of the HpSA positive samples tested. In our opinion, this provides evidence that positive HpSA tests in newborn infants are not likely due to cross-reactions i.e. with perinatal transitory antigens, but probably represent presence of *H. pylori* bacteria in the stool. The difficulties of performing PCR in stool-extracted DNA are well recognized, and arise due to the presence of PCR inhibitors and local variations in DNA density within the stool. This explains the divergence between the HpSA- and the PCR findings.

5.3.6 *H. pylori* in neonates - transmission from mother to child?

The findings in paper I indicate that a great proportion of infants encounter *H. pylori* in the neonatal period. The fact that *H. pylori* antigen in the present study was detected in only 10% of the infants delivered by cesarean section compared to 59% of infants born by vaginal births, suggests that transmission from mother to child probably occurs during the baby's passage through the birth canal. As the *H. pylori* detection rate in infants dropped after the perinatal period and remained low for all other age groups in infancy and childhood, the encounter is not likely to result in a persistent infection in individuals of normal health. The luminal content of the fetal gastrointestinal tract consists of sterile meconium which is colonized with bacteria shortly after birth. The timing and composition of bacterial colonization has been shown to be determined by the mode of delivery and maternal vaginal microbes have been detected in the stool of neonates born by vaginal births[175]. A Pubmed search reveals that efforts to investigate the development of the normal infant microbiota have been rather modest in later years. However, Palmer et al recently collected repeated stool samples of infants from birth to one year of age, and performed microarray to detect bacterial rRNA[176]. They showed that great changes occur in the intestinal microbiota in most individuals in the first months of life.

5.4 Clinical significance of H. pylori in newborn infants

In paper I we concluded that *H. pylori* is probably transmitted from mother to child at the time of birth. However, based on the study findings, this would indicate that more than half of the Norwegian mothers are carriers of *H. pylori*. Data from the HUNT study suggest that only 10-15% of Norwegian women at a fertile age are *H. pylori* positive by serology[177]. Thus a large proportion of women either do not show an immune response against their *H. pylori* infection, or the women acquire their *H. pylori* infection in pregnancy and/or do not develop an immune response. Interestingly, studies have shown that *H. pylori* (re-)infection in pregnancy may occur and an association between hyperemesis gravidarum has been found[178]. There is also emerging evidence that *H. pylori* might enter into a viable, but not culturable state – a coccoid form which has been observed in colonies suffering stress and nutrient depletion in laboratory conditons[179;180].We speculate that *H. pylori* organisms

in some form are introduced to infants shortly after birth and are "flushed" through the gastrointestinal tract within the first week(s) of life in healthy individuals. The existence of *H. pylori* bacteria in a viable, non-virulent form in the gastro-intestinal tract, could explain the obvious incongruity between the HpSA test findings in newborn infants and the predicted *H. pylori* prevalence in their mothers. A follow-up study testing both mothers and infants may disclose the transmission pathway, and is in progress.

5.5 Clinical significance of H. pylori infection in SIDS

The drop in SIDS rate following the intervention programs directed against prone sleeping and smoking in pregnancy, predominantly involved young infants between 1-5 months of age whereas older infants were less affected[52]. In paper II we show that the difference in *H. pylori* infection rate between SIDS and controls is predominantly seen in infants aged between 1-5 months. Furthermore, in paper III we present that infants in this age group express low surfactant protein A in their lungs. There is now substantial evidence that in a large proportion of SIDS cases (~50%) the death mechanism may be induced by disturbances in the immunological homeostasis caused by a combination of genetic predisposition and a trigger event at this vulnerable stage in development [13;38;181]. *H. pylori* may represent a biomarker to detect such cases of SIDS. We speculate that *H. pylori* infection contribute to the microbial load, and trigger the death mechanism in SIDS (Figure 8).

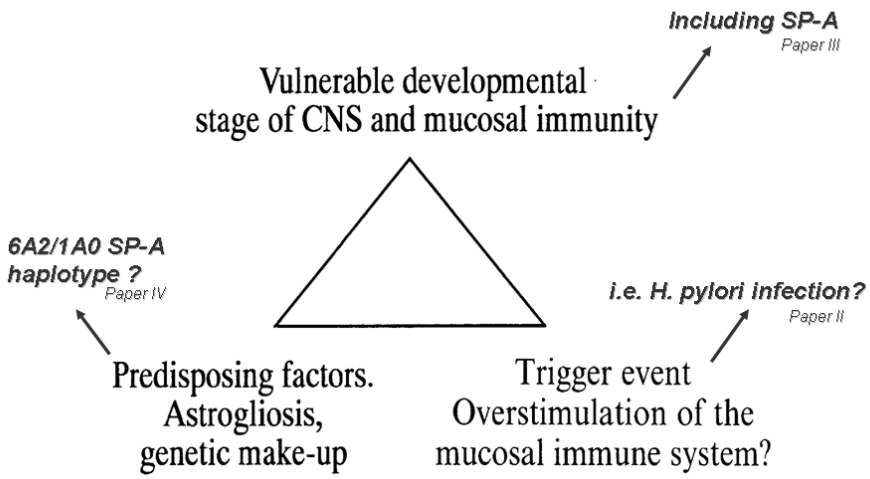

Figure 8. The Fatal triangle supplemented with the results of the present thesis.

From Rognum and Saugstad. Biochemical and immunological studies in SIDS victims. Clues to understanding the death mechanism. Acta Paediatr.Suppl 1993

5.6 Collectins –significance for SIDS?

In paper III we present that SP-A expression drops in the first months after birth, corresponding with the classical age peak for SIDS. In what manner this phenomenon is

related to SIDS other than adding to the burden of vulnerability in the first months of life (Figure 8), remain unidentified. SP-A plays a structural role within the surfactant phospholipid[127] and, hypothetically, a deficiency in SP-A function would explain the findings of partial atelectasis which is a common feature in infant deaths at this age. However, we were unable to find an association between observed atelectasis and high or low SP-A expression. Further studies are needed to elucidate the role of the dip in SP-A expression in the post-natal period. Evaluating the amount of SP-A transcripts by Real-time PCR technology would be a valuable contribution, but is difficult to perform on material obtained at autopsy due to rapid autolysis of mRNA after death.

Recent studies have shown that Surfactant protein D is involved in the immune response to *H. pylori*[108;182]. Both SP-A and SP-D are powerful modulators of the immune response[88]. A deficiency in these innate components has not yet been observed in humans, but may in theory contribute to the immunological disturbance in SIDS. A state of clinical deficiency in the third collectin, MBL, is fairly common (~1% of the population) and is associated with increased susceptibility to infections[132]. The aim of the study presented in paper IV was to elucidate whether genetic variants of the collectins could be associated with SIDS. We report that no significant associations with SIDS are found. However, the study is, like several gene association studies, limited by the fact that only the currently well-characterized polymorphisms in the coding exons were investigated. The traditional PCR-methodology used is accurate, but time-consuming. New technology enabling rapid detection of polymorphic sites generates new opportunities for genetic research, and the possibility of detecting yet unidentified gene variations.

6 Conclusions

1. *Helicobacter pylori* stool antigen is detected in the primary meconium discharge of 60% of Norwegian newborn infants delivered vaginally compared to 10% of infants delivered by cesarean section. Transmission of *H. pylori* most likely occurs from mother to child related to the baby's passage through the birth canal. After the neonatal period the detection rate in healthy Norwegian infants is low (~5%), indicating a transient infection in most cases.

2. *H. pylori* infection in post-natal infants, confirmed by the HpSA test and by immune staining of gastric tisse, is associated with SIDS and sudden deaths in infancy due to infectious disease, particularly for infants between 1 and 5 months of age. Elevated interleukin-6 levels in cerebrospinal fluid were found in *H. pylori* positive SIDS victims, indicating a contribution to the "load" of triggering events in SIDS.

3. Abundant surfactant protein A (SP-A) expression is demonstrated in neonates born to term. Low SP-A expression is observed in infants of post-natal age between 2 weeks and 5 months of age, coinciding with the critical age peak for SIDS, thus contributing to the crtical developmental stage.

4. The common SP-A haplotype 6A2/1A0 tends to be associated with low protein expression in post-natal infants. Genetic variants of surfactant protein D (SP-D) determined by polymorphisms at aa11 and aa160 are not associated with high/low protein expression. Genetic variants of SP-A, SP-D or mannose-binding lectin, MBL, are not associated with SIDS.

7 REFERENCES

(1) Old Testament. Bible. **First book of Kings** ed. 2007.
(2) Norvenius G. Is SIDS a new phenomenon? In: Rognum TO, editor. Sudden Infant Death Syndrome. New Trends in the Nineties. Oslo: Scandinavian University Press; 1995. 11-14.
(3) Paltauf A. Plötzlichen Thymustod. Wien Klin Wochenschr 1889; II:877.
(4) Valdes-Dapena M. A half century of progress: The evolution of SIDS research. In: Rognum TO, editor. Sudden Infant Death Syndrome. New Trends in the Nineties. Oslo: Scandinavian University Press; 1995. 3-10.
(5) Limerick SR. Sudden infant death in historical perspective. J Clin Pathol 1992; 45(11 Suppl):3-6.
(6) Spain DM, Bradess VA, Greenblatt IJ. Possible factor in sudden and unexpected death during infancy. J Am Med Assoc 1954; 156(3):246-247.
(7) Valdes-Dapena MA, Eichman MF, Ziskin L. Sudden and unexpected death in infants. I. Gamma globulin levels in the serum. J Pediatr 1963; 63:290-294.
(8) Cordner SM, Willinger M. The definition of the sudden infant death syndrome. In: Rognum TO, editor. Sudden Infant Death Syndrome. New Trends in the Nineties. Oslo: Scandinavian University Press; 1995. 17-20.
(9) Beckwith JB. Discussion of terminology and definiton of the sudden infant death syndrome. In: Bergman AB, Beckwith JB, Ray CG, eds Sudden infant death syndrome Proceedings of the Second International Conference on the Causes of Sudden Death in Infants Seattle: University of Washington Press 1970;14-22.
(10) Krous HF, Beckwith JB, Byard RW, Rognum TO, Bajanowski T, Corey T et al. Sudden infant death syndrome and unclassified sudden infant deaths: a definitional and diagnostic approach. Pediatrics 2004; 114(1):234-238.
(11) Rognum TO. Definition and pathologic features. In: Byard RW, Krous HF, editors. Sudden infant death syndrome. Problems, progress and possibilites. 1 ed. London: Arnold, Hodder Headline Group; 2001. 4-30.
(12) Stray-Pedersen A, Rognum TO. Evaluation of circumstances and clinical history in SUDI. Scand J of Forens Sci 2004; 10(3/4):77-79.
(13) Rognum TO, Byard RW. Sudden Infant Death Syndrome, etiology and epidemiology. In: Payne-James J, Byard RW, Corey T, Henderson C, editors. Encyclopedia of Forensic and Legal Medicine. 2 ed. Amsterdam: Elsevier / Academic Press; 2005. 117-127.
(14) Steinschneider A. Prolonged apnea and the sudden infant death syndrome: clinical and laboratory observations. Pediatrics 1972; 50(4):646-654.
(15) Schwartz PJ. Cardiac sympathetic innervation and the sudden infant death syndrome. A possible pathogenetic link. Am J Med 1976; 60(2):167-172.
(16) Schwartz PJ, Stramba-Badiale M, Segantini A, Austoni P, Bosi G, Giorgetti R et al. Prolongation of the QT interval and the sudden infant death syndrome. N Engl J Med 1998; 338(24):1709-1714.
(17) Arnestad M, Crotti L, Rognum TO, Insolia R, Pedrazzini M, Ferrandi C et al. Prevalence of long-QT syndrome gene variants in sudden infant death syndrome. Circulation 2007; 115(3):361-367.
(18) Kahn A, Riazi J, Blum D. Oculocardiac reflex in near miss for sudden infant death syndrome infants. Pediatrics 1983; 71(1):49-52.
(19) Kelly DH, Golub H, Carley D, Shannon DC. Pneumograms in infants who subsequently died of sudden infant death syndrome. J Pediatr 1986; 109(2):249-254.
(20) Kluge KA, Harper RM, Schechtman VL, Wilson AJ, Hoffman HJ, Southall DP. Spectral analysis assessment of respiratory sinus arrhythmia in normal infants and infants who subsequently died of sudden infant death syndrome. Pediatr Res 1988; 24(6):677-682.
(21) Kahn A, Groswasser J, Sottiaux M, Rebuffat E, Franco P, Dramaix M. Prone or supine body position and sleep characteristics in infants. Pediatrics 1993; 91(6):1112-1115.
(22) Naeye RL. Brain-stem and adrenal abnormalities in the sudden-infant-death syndrome. Am J Clin Pathol 1976; 66(3):526-530.
(23) Summers CG, Parker JC, Jr. The brain stem in sudden infant death syndrome. A postmortem survey. Am J Forensic Med Pathol 1981; 2(2):121-127.
(24) Kinney HC, Burger PC, Harrell FE, Jr., Hudson RP, Jr. 'Reactive gliosis' in the medulla oblongata of victims of the sudden infant death syndrome. Pediatrics 1983; 72(2):181-187.
(25) Becker LE. Neural maturational delay as a link in the chain of events leading to SIDS. Can J Neurol Sci 1990; 17(4):361-371.

(26) Takashima S, Becker LE. Developmental abnormalities of medullary "respiratory centers" in sudden infant death syndrome. Exp Neurol 1985; 90(3):580-587.
(27) Rognum TO, Saugstad OD, Oyasaeter S, Olaisen B. Elevated levels of hypoxanthine in vitreous humor indicate prolonged cerebral hypoxia in victims of sudden infant death syndrome. Pediatrics 1988; 82(4):615-618.
(28) Tonkin S. Sudden infant death syndrome: hypothesis of causation. Pediatrics 1975; 55(5):650-661.
(29) Tonkin S. On listening to parents: the sudden infant death syndrome over 25 years. Pediatrics 1996; 97(6 Pt 1):896-897.
(30) de Jonge GA, Engelberts AC, Koomen-Liefting AJ, Kostense PJ. Cot death and prone sleeping position in The Netherlands. BMJ 1989; 298(6675):722.
(31) Mitchell EA. Cot death: should the prone sleeping position be discouraged? J Paediatr Child Health 1991; 27(6):319-321.
(32) Markestad T. [Sleeping position and crib death in Norway]. Tidsskr Nor Laegeforen 1992; 112(11):1427-1429.
(33) Fleming PJ, Gilbert R, Azaz Y, Berry PJ, Rudd PT, Stewart A et al. Interaction between bedding and sleeping position in the sudden infant death syndrome: a population based case-control study. BMJ 1990; 301(6743):85-89.
(34) Irgens LM, Markestad T, Baste V, Schreuder P, Skjaerven R, Oyen N. Sleeping position and sudden infant death syndrome in Norway 1967-91. Arch Dis Child 1995; 72(6):478-482.
(35) Beal SM, Finch CF. An overview of retrospective case-control studies investigating the relationship between prone sleeping position and SIDS. J Paediatr Child Health 1991; 27(6):334-339.
(36) Guntheroth WG, Spiers PS. The triple risk hypotheses in sudden infant death syndrome. Pediatrics 2002; 110(5):e64.
(37) Vege A, Rognum TO. Sudden infant death syndrome, infection and inflammatory responses. FEMS Immunol Med Microbiol 2004; 42(1):3-10.
(38) Rognum TO, Saugstad OD. Biochemical and immunological studies in SIDS victims. Clues to understanding the death mechanism. Acta Paediatr Suppl 1993; 82 Suppl 389:82-85.
(39) Gregersen M, Rajs J, Laursen H, Baandrup U, Frederiksen P, Gidlund E. Pathologic criteria for the Nordic study of SIDS. In: Rognum TO (ed) Sudden Infant Death Syndrome New Trends in the Nineties Scandinavian University Press, Oslo 1995;50-58.
(40) Nordic Council of Ministers. Sudden infant death in the Nordic countries. Results of the Nordic study of Sudden Infant Death Syndrome 1990-1996. Copenhagen, Denmark: Tema Nord; 1997.
(41) Rognum TO, Arnestad M, Bajanowski T, Banner J, Blair P, Borthne A et al. Consensus on Diagnostic Criteria for the Exclusion of SIDS. Scand J of Forens Sci 2003; 9(3/4):62-73.
(42) Byard RW, Krous HF. Differential diagnosis of sudden infant death. In: Byard RW, Krous HF, editors. Sudden infant death syndrome. Problems, progress and possibilites. 1 ed. London: Arnold, Hodder Headline Group; 2001. 209-227.
(43) Vege A, Rognum TO. Use of new Nordic criteria for classification of SIDS to re-evaluate diagnoses of sudden unexpected infant death in the Nordic countries. Acta Paediatr 1997; 86(4):391-396.
(44) Stray-Pedersen A, Arnestad M, Opdal S, Vege A, Rognum TO, Byard R. Globally accepted definition and diagnostic criteria - crucial for solving the SIDS engima. Scand J of Forens Sci 2004; 10(3/4):70-71.
(45) Bajanowski T, Vege A, Byard RW, Krous HF, Arnestad M, Bachs L et al. Sudden infant death syndrome (SIDS)-Standardised investigations and classification: Recommendations. Forensic Sci Int 2006.
(46) de Lange C, Stray-Pedersen A, Borthne A, Rognum TO. Radiological investigation of SUDI. Scand J of Forens Sci 2004; 10(3/4):74.
(47) Fleming PJ, Blair PS, Sidebotham PD, Hayler T. Investigating sudden unexpected deaths in infancy and childhood and caring for bereaved families: an integrated multiagency approach. BMJ 2004; 328(7435):331-334.
(48) Sveum L, Rognum TO, Vege A, Arnestad M, Stray-Pedersen A. Barnedødsårsaksprosjektet. Rapport til Riksadvokaten med forslag til fremtidig organisering av undersøkelser ved plutselig uventet død i sped- og småbarnsalder 2004.
(49) Blair PS, Sidebotham P, Berry PJ, Evans M, Fleming PJ. Major epidemiological changes in sudden infant death syndrome: a 20-year population-based study in the UK. Lancet 2006; 367(9507):314-319.
(50) Carpenter RG, Irgens LM, Blair PS, England PD, Fleming P, Huber J et al. Sudden unexplained infant death in 20 regions in Europe: case control study. Lancet 2004; 363(9404):185-191.

(51) Arnestad M, Andersen M, Vege A, Rognum TO. Changes in the epidemiological pattern of sudden infant death syndrome in southeast Norway, 1984-1998: implications for future prevention and research. Arch Dis Child 2001; 85(2):108-115.
(52) Vege A, Rognum TO, Opdal SH. SIDS--changes in the epidemiological pattern in eastern Norway 1984-1996. Forensic Sci Int 1998; 93(2-3):155-166.
(53) Gilbert RE, Wigfield RE, Fleming PJ, Berry PJ, Rudd PT. Bottle feeding and the sudden infant death syndrome. BMJ 1995; 310(6972):88-90.
(54) Stray-Pedersen A, Arnestad M, Vege A, Sveum L, Rognum TO. [Bed sharing as a risk factor for sudden infant death syndrome.]. Tidsskr Nor Laegeforen 2005; 125(21):2919-2921.
(55) Kemp JS, Nelson VE, Thach BT. Physical properties of bedding that may increase risk of sudden infant death syndrome in prone-sleeping infants. Pediatr Res 1994; 36(1 Pt 1):7-11.
(56) Kemp JS, Kowalski RM, Burch PM, Graham MA, Thach BT. Unintentional suffocation by rebreathing: a death scene and physiologic investigation of a possible cause of sudden infant death. J Pediatr 1993; 122(6):874-880.
(57) Ponsonby AL, Dwyer T, Gibbons LE, Cochrane JA, Wang YG. Factors potentiating the risk of sudden infant death syndrome associated with the prone position. N Engl J Med 1993; 329(6):377-382.
(58) Molony N, Blackwell CC, Busuttil A. The effect of prone posture on nasal temperature in children in relation to induction of staphylococcal toxins implicated in sudden infant death syndrome. FEMS Immunol Med Microbiol 1999; 25(1-2):109-113.
(59) Horne RS, Ferens D, Watts AM, Vitkovic J, Lacey B, Andrew S et al. The prone sleeping position impairs arousability in term infants. J Pediatr 2001; 138(6):811-816.
(60) Mitchell EA. Smoking: The next major and modifiable risk factor. In: Rognum TO, editor. Sudden Infant Death Syndrome. New Trends in the Nineties. Oslo: Scandinavian University Press; 1995. 114-118.
(61) White M, Beckett M, O'Regan M, Matthews T. The effect of maternal smoking in pregnancy on autonomic function in infants. In: Rognum TO, editor. Sudden Infant Death Syndrome. New Trends in the Nineties. Oslo: Scandinavian University Press; 1995. 174-176.
(62) Greenough A, Yuksel B, Cheeseman P. Effect of in utero growth retardation on lung function at follow-up of prematurely born infants. Eur Respir J 2004; 24(5):731-733.
(63) Horne RS, Parslow PM, Harding R. Postnatal development of ventilatory and arousal responses to hypoxia in human infants. Respir Physiol Neurobiol 2005; 149(1-3):257-271.
(64) Milerad J, Vege A, Opdal SH, Rognum TO. Objective measurements of nicotine exposure in victims of sudden infant death syndrome and in other unexpected child deaths. J Pediatr 1998; 133(2):232-236.
(65) Froen JF, Akre H, Stray-Pedersen B, Saugstad OD. Prolonged apneas and hypoxia mediated by nicotine and endotoxin in piglets. Biol Neonate 2002; 81(2):119-125.
(66) Horne RS, Franco P, Adamson TM, Groswasser J, Kahn A. Influences of maternal cigarette smoking on infant arousability. Early Hum Dev 2004; 79(1):49-58.
(67) Tappin D, Ecob R, Brooke H. Bedsharing, roomsharing, and sudden infant death syndrome in Scotland: a case-control study. J Pediatr 2005; 147(1):32-37.
(68) Blair PS, Ball HL. The prevalence and characteristics associated with parent-infant bed-sharing in England. Arch Dis Child 2004; 89(12):1106-1110.
(69) McKenna JJ, McDade T. Why babies should never sleep alone: A review of the co-sleeping controversy in relation to SIDS, bedsharing and breast feeding. Paediatric Respiratory Reviews 2005; 6(2):134-152.
(70) Hull D, McArthur AJ, Pritchard K, Oldham D. Individual variation in sleeping metabolic rates in infants. Arch Dis Child 1996; 75(4):288-291.
(71) Petersen SA, Pratt C, Wailoo MP. Relations between the development of patterns of sleeping heart rate and body temperature in infants. Arch Dis Child Fetal Neonatal Ed 2001; 85(2):F133-F136.
(72) Arkell S, Blair P, Henderson J, Fleming P. Is the mattress important in helping babies keep warm?--Paradoxical effects of a sleeping surface with negligible thermal resistance. Acta Paediatr 2007; 96(2):199-205.
(73) Werne J. Postmortem evidence of acute infections in unexpected death in infancy. Am J Pathol 1942; 18:756-761.
(74) Lindgren C, Milerad J, Lagercrantz H. Sudden infant death and prevalence of whooping cough in the Swedish and Norwegian communities. Eur J Pediatr 1997; 156(5):405-409.
(75) Lindgren C. Respiratory syncytial virus and the sudden infant death syndrome. Acta Paediatr Suppl 1993; 82 Suppl 389:67-69.
(76) Samuels M. Viruses and sudden infant death. Paediatr Respir Rev 2003; 4(3):178-183.

(77) Bajanowski T, Rolf B, Jorch G, Brinkmann B. Detection of RNA viruses in sudden infant death (SID). Int J Legal Med 2003; 117(4):237-240.
(78) Blackwell CC, Gordon AE, James VS, MacKenzie DA, Mogensen-Buchanan M, El Ahmer OR et al. The role of bacterial toxins in sudden infant death syndrome (SIDS). Int J Med Microbiol 2002; 291(6-7):561-570.
(79) Blackwell CC, Weir DM, Busuttil A. A microbiologcial perspective. In: Byard RW, Krous HF, editors. Sudden infant death syndrome. Problems, progress and possibilites. 1 ed. London: Arnold, Hodder Headline Group; 2001. 182-208.
(80) Stoltenberg L, Saugstad OD, Rognum TO. Sudden infant death syndrome victims show local immunoglobulin M response in tracheal wall and immunoglobulin A response in duodenal mucosa. Pediatr Res 1992; 31(4 Pt 1):372-375.
(81) Stoltenberg L, Vege A, Saugstad OD, Rognum TO. Changes in the concentration and distribution of immunoglobulin-producing cells in SIDS palatine tonsils. Pediatr Allergy Immunol 1995; 6(1):48-55.
(82) Vege A, Rognum TO, Anestad G. IL-6 cerebrospinal fluid levels are related to laryngeal IgA and epithelial HLA-DR response in sudden infant death syndrome. Pediatr Res 1999; 45(6):803-809.
(83) Thrane PS, Rognum TO, Brandtzaeg P. Up-regulated epithelial expression of HLA-DR and secretory component in salivary glands: reflection of mucosal immunostimulation in sudden infant death syndrome. Pediatr Res 1994; 35(5):625-628.
(84) Thrane PS, Rognum TO, Brandtzaeg P. Ontogenesis of the secretory immune system and innate defence factors in human parotid glands. Clin Exp Immunol 1991; 86(2):342-348.
(85) Stoltenberg L, Thrane PS, Rognum TO. Development of immune response markers in the trachea in the fetal period and the first year of life. Pediatr Allergy Immunol 1993; 4(1):13-19.
(86) Rognum TO, Thrane S, Stoltenberg L, Vege A, Brandtzaeg P. Development of intestinal mucosal immunity in fetal life and the first postnatal months. Pediatr Res 1992; 32(2):145-149.
(87) Koch A, Melbye M, Sorensen P, Homoe P, Madsen HO, Molbak K et al. Acute respiratory tract infections and mannose-binding lectin insufficiency during early childhood. JAMA 2001; 285(10):1316-1321.
(88) Holmskov U, Thiel S, Jensenius JC. Collectins and Ficolins: Humoral Lectins of the Innate Immune Defense. Annu Rev Immunol 2003; 21:547-578.
(89) Guntheroth WG. Interleukin-1 as intermediary causing prolonged sleep apnea and SIDS during respiratory infections. Med Hypotheses 1989; 28(2):121-123.
(90) Vege A, Rognum TO, Scott H, Aasen AO, Saugstad OD. SIDS cases have increased levels of interleukin-6 in cerebrospinal fluid. Acta Paediatr 1995; 84(2):193-196.
(91) Moscovis SM, Gordon AE, Al Madani OM, Gleeson M, Scott RJ, Roberts-Thomson J et al. IL6 G-174C associated with sudden infant death syndrome in a Caucasian Australian cohort. Hum Immunol 2006; 67(10):819-825.
(92) Opdal SH, Rognum TO. The IL6 -174G/C polymorphism and sudden infant death syndrome. Hum Immunol 2007; 68(6):541-543.
(93) Opdal SH, Opstad A, Vege A, Rognum TO. IL-10 gene polymorphisms are associated with infectious cause of sudden infant death. Hum Immunol 2003; 64(12):1183-1189.
(94) Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1984; 1(8390):1311-1315.
(95) Goodwin CS, Armstrong JA. Microbiological aspects of Helicobacter pylori (Campylobacter pylori). Eur J Clin Microbiol Infect Dis 1990; 9(1):1-13.
(96) Mitchell HM. The epidemiology of Helicobacter pylori. Curr Top Microbiol Immunol 1999; 241:11-30.
(97) Granstrom M, Tindberg Y, Blennow M. Seroepidemiology of Helicobacter pylori infection in a cohort of children monitored from 6 months to 11 years of age. J Clin Microbiol 1997; 35(2):468-470.
(98) Rehnberg-Laiho L, Rautelin H, Valle M, Kosunen TU. Persisting Helicobacter antibodies in Finnish children and adolescents between two and twenty years of age. Pediatr Infect Dis J 1998; 17(9):796-799.
(99) Andersen LP. Colonization and Infection by Helicobacter pylori in Humans. Helicobacter 2007; 12 Suppl 2:12-15.
(100) Thomas JE, Dale A, Harding M, Coward WA, Cole TJ, Weaver LT. Helicobacter pylori colonization in early life. Pediatr Res 1999; 45(2):218-223.
(101) Casswall T. Helicobacter pylori infection, ulcer disease, and gastritis in children. The Mead Johnson Nutritionals Journals of Pediatrics Review Series 2002;(3).

(102) Kivi M, Tindberg Y. Helicobacter pylori occurrence and transmission: a family affair? Scand J Infect Dis 2006; 38(6-7):407-417.
(103) Yilmaz E, Dogan Y, Gurgoze MK, Unal S. Seroprevalence of Helicobacter pylori infection among children and their parents in eastern Turkey. J Paediatr Child Health 2002; 38(2):183-186.
(104) Kumagai T, Malaty HM, Graham DY, Hosogaya S, Misawa K, Furihata K et al. Acquisition versus loss of Helicobacter pylori infection in Japan: results from an 8-year birth cohort study. J Infect Dis 1998; 178(3):717-721.
(105) Andersen LP. Colonization and Infection by Helicobacter pylori in Humans. Helicobacter 2007; 12 Suppl 2:12-15.
(106) Andersen LP, Dorland A, Karacan H, Colding H, Nilsson HO, Wadstrom T et al. Possible clinical importance of the transformation of Helicobacter pylori into coccoid forms. Scand J Gastroenterol 2000; 35(9):897-903.
(107) Cellini L, Grande R, Campli ED, Traini T, Giulio MD, Lannutti SN et al. Dynamic colonization of Helicobacter pylori in human gastric mucosa. Scand J Gastroenterol 2007;1-8.
(108) Murray E, Khamri W, Walker MM, Eggleton P, Moran AP, Ferris JA et al. Expression of surfactant protein D in the human gastric mucosa and during Helicobacter pylori infection. Infect Immun 2002; 70(3):1481-1487.
(109) Khamri W, Worku ML, Anderson AE, Walker MM, Hawgood S, Reid KB et al. Helicobacter infection in the surfactant protein D-deficient mouse. Helicobacter 2007; 12(2):112-123.
(110) Zhao Y, Yokota K, Ayada K, Yamamoto Y, Okada T, Shen L et al. Helicobacter pylori heat-shock protein 60 induces interleukin-8 via a Toll-like receptor (TLR)2 and mitogen-activated protein (MAP) kinase pathway in human monocytes. J Med Microbiol 2007; 56(Pt 2):154-164.
(111) Versalovic J. Helicobacter pylori. Pathology and diagnostic strategies. Am J Clin Pathol 2003; 119(3):403-412.
(112) Egan BJ, Holmes K, O'Connor HJ, O'Morain CA. Helicobacter pylori Gastritis, the Unifying Concept for Gastric Diseases. Helicobacter 2007; 12 Suppl 2:39-44.
(113) Veres G, Pehlivanoglu E. Helicobacter pylori infection in pediatrics. Helicobacter 2007; 12 Suppl 1:38-44.
(114) Franceschi F, Gasbarrini A. Helicobacter pylori and extragastric diseases. Best Pract Res Clin Gastroenterol 2007; 21(2):325-334.
(115) Pattison CP, Marshall BJ. Proposed link between Helicobacter pylori and sudden infant death syndrome. Med Hypotheses 1997; 49(5):365-369.
(116) Kerr JR, Al Khattaf A, Barson AJ, Burnie JP. An association between sudden infant death syndrome (SIDS) and Helicobacter pylori infection. Arch Dis Child 2000; 83(5):429-434.
(117) Elitsur Y, Btriest W, Sabet Z, Neace C, Jiang C, Thomas E. Is sudden infant death syndrome associated with Helicobacter pylori infection in children? Helicobacter 2000; 5(4):227-231.
(118) Ho GY, Windsor HM, Snowball B, Marshall BJ. Helicobacter pylori is not the cause of sudden infant death syndrome (SIDS). Am J Gastroenterol 2001; 96(12):3288-3294.
(119) Sullivan LC, Daniels CB, Phillips ID, Orgeig S, Whitsett JA. Conservation of surfactant protein A: evidence for a single origin for vertebrate pulmonary surfactant. J Mol Evol 1998; 46(2):131-138.
(120) Rooney SA. Lung surfactant. Environ Health Perspect 1984; 55:205-226.
(121) Liu M, Wang L, Li E, Enhorning G. Pulmonary surfactant given prophylactically alleviates an asthma attack in guinea-pigs. Clin Exp Allergy 1996; 26(3):270-275.
(122) De Sanctis GT, Tomkiewicz RP, Rubin BK, Schurch S, King M. Exogenous surfactant enhances mucociliary clearance in the anaesthetized dog. Eur Respir J 1994; 7(9):1616-1621.
(123) Talbert DG, Southall DP. A bimodal form of alveolar behaviour induced by a defect in lung surfactant--a possible mechanism for sudden infant death syndrome. Lancet 1985; 1(8431):727-728.
(124) Perez-Gil J. Lipid-protein interactions of hydrophobic proteins SP-B and SP-C in lung surfactant assembly and dynamics. Pediatr Pathol Mol Med 2001; 20(6):445-469.
(125) Whitsett JA, Wert SE, Trapnell BC. Genetic disorders influencing lung formation and function at birth. Hum Mol Genet 2004; 13 Spec No 2:R207-R215.
(126) Ochs M, Johnen G, Muller KM, Wahlers T, Hawgood S, Richter J et al. Intracellular and intraalveolar localization of surfactant protein A (SP-A) in the parenchymal region of the human lung. Am J Respir Cell Mol Biol 2002; 26(1):91-98.
(127) Wright JR. Immunomodulatory functions of surfactant. Physiol Rev 1997; 77(4):931-962.
(128) Sorensen GL, Husby S, Holmskov U. Surfactant protein A and surfactant protein D variation in pulmonary disease. Immunobiology 2007; 212(4-5):381-416.
(129) Madsen J, Tornoe I, Nielsen O, Koch C, Steinhilber W, Holmskov U. Expression and Localization of Lung Surfactant Protein A in Human Tissues. Am J Respir Cell Mol Biol 2003; 29(5):591-597.

(130) Madsen J, Kliem A, Tornoe I, Skjodt K, Koch C, Holmskov U. Localization of lung surfactant protein D on mucosal surfaces in human tissues. J Immunol 2000; 164(11):5866-5870.
(131) Crouch E, Persson A, Chang D, Heuser J. Molecular structure of pulmonary surfactant protein D (SP-D). J Biol Chem 1994; 269(25):17311-17319.
(132) Garred P, Strom J, Quist L, Taaning E, Madsen HO. Association of mannose-binding lectin polymorphisms with sepsis and fatal outcome, in patients with systemic inflammatory response syndrome. J Infect Dis 2003; 188(9):1394-1403.
(133) McCormack FX, Gibbons R, Ward SR, Kuzmenko A, Wu H, Deepe GS, Jr. Macrophage-independent fungicidal action of the pulmonary collectins. J Biol Chem 2003; 278(38):36250-36256.
(134) LeVine AM, Whitsett JA, Gwozdz JA, Richardson TR, Fisher JH, Burhans MS et al. Distinct effects of surfactant protein A or D deficiency during bacterial infection on the lung. J Immunol 2000; 165(7):3934-3940.
(135) LeVine AM, Whitsett JA, Gwozdz JA, Richardson TR, Fisher JH, Burhans MS et al. Distinct effects of surfactant protein A or D deficiency during bacterial infection on the lung. J Immunol 2000; 165(7):3934-3940.
(136) Jounblat R, Kadioglu A, Iannelli F, Pozzi G, Eggleton P, Andrew PW. Binding and agglutination of Streptococcus pneumoniae by human surfactant protein D (SP-D) vary between strains, but SP-D fails to enhance killing by neutrophils. Infect Immun 2004; 72(2):709-716.
(137) LeVine AM, Whitsett JA, Gwozdz JA, Richardson TR, Fisher JH, Burhans MS et al. Distinct effects of surfactant protein A or D deficiency during bacterial infection on the lung. J Immunol 2000; 165(7):3934-3940.
(138) Sicinschi LA, Correa P, Bravo LE, Schneider BG. Detection and typing of Helicobacter pylori cagA/vacA genes by radioactive, one-step polymerase chain reaction in stool samples from children. J Microbiol Methods 2003; 52(2):197-207.
(139) Gramley WA, Asghar A, Frierson HF, Jr., Powell SM. Detection of Helicobacter pylori DNA in fecal samples from infected individuals. J Clin Microbiol 1999; 37(7):2236-2240.
(140) Pantelidis P, Lagan AL, Davies JC, Welsh KI, du Bois RM. A single round PCR method for genotyping human surfactant protein (SP)-A1, SP-A2 and SP-D gene alleles. Tissue Antigens 2003; 61(4):317-321.
(141) DiAngelo S, Lin Z, Wang G, Phillips S, Ramet M, Luo J et al. Novel, non-radioactive, simple and multiplex PCR-cRFLP methods for genotyping human SP-A and SP-D marker alleles. Dis Markers 1999; 15(4):269-281.
(142) Ramet M, Haataja R, Marttila R, Hamalainen AM, Knip M, Hallman M. Human surfactant protein--A gene locus for genetic studies in the Finnish population. Dis Markers 2000; 16(3-4):119-124.
(143) Valdes-Dapena M. Sudden infant death syndrome. Morphology update for forensic pathologists--1985. Forensic Sci Int 1986; 30(2-3):177-186.
(144) Filiano JJ, Kinney HC. A perspective on neuropathologic findings in victims of the sudden infant death syndrome: the triple-risk model. Biol Neonate 1994; 65(3-4):194-197.
(145) Kahn A, Groswasser J, Kelmanson I. Risk factors for SIDS: Risk factors for ALTE? From epidemiology to physiology. In: Rognum TO, editor. Sudden Infant Death Syndrome. New Trends in the Nineties. Oslo: Scandinavian University Press; 1995. 132-137.
(146) Maehlen J, Olsson T, Zachau A, Klareskog L, Kristensson K. Local enhancement of major histocompatibility complex (MHC) class I and II expression and cell infiltration in experimental allergic encephalomyelitis around axotomized motor neurons. J Neuroimmunol 1989; 23(2):125-132.
(147) Takashima S, Armstrong D, Becker L, Bryan C. Cerebral hypoperfusion in the sudden infant death syndrome? Brainstem gliosis and vasculature. Ann Neurol 1978; 4(3):257-262.
(148) Opdal SH, Rognum TO. The sudden infant death syndrome gene: does it exist? Pediatrics 2004; 114(4):e506-e512.
(149) Summers AM, Summers CW, Drucker DB, Hajeer AH, Barson A, Hutchinson IV. Association of IL-10 genotype with sudden infant death syndrome. Hum Immunol 2000; 61(12):1270-1273.
(150) Moscovis SM, Gordon AE, Al Madani OM, Gleeson M, Scott RJ, Roberts-Thomson J et al. Interleukin-10 and sudden infant death syndrome. FEMS Immunol Med Microbiol 2004; 42(1):130-138.
(151) Dashash M, Pravica V, Hutchinson IV, Barson AJ, Drucker DB. Association of sudden infant death syndrome with VEGF and IL-6 gene polymorphisms. Hum Immunol 2006; 67(8):627-633.
(152) Schneider PM, Wendler C, Riepert T, Braun L, Schacker U, Horn M et al. Possible association of sudden infant death with partial complement C4 deficiency revealed by post-mortem DNA typing of HLA class II and III genes. Eur J Pediatr 1989; 149(3):170-174.
(153) Opdal SH, Vege A, Saugstad OD, Rognum TO. Is partial deletion of the complement C4 genes associated with sudden infant death? Eur J Pediatr 1994; 153(4):287-290.

(154) Opdal SH, Vege A, Stave AK, Rognum TO. The complement component C4 in sudden infant death. Eur J Pediatr 1999; 158(3):210-212.
(155) Fatemi A, Item C, Stockler-Ipsiroglu S, Ipsiroglu O, Sperl W, Patsch W et al. Sudden infant death: no evidence for linkage to common polymorphisms in the uncoupling protein-1 and the beta3-adrenergic receptor genes. Eur J Pediatr 2002; 161(6):337-339.
(156) Roman R, Lopez P, Johnson MC, Boric MA, Gallo M, Ponce C et al. Sudden infant death syndrome and activating gnas1 gene mutations. Fetal Pediatr Pathol 2007; 26(4):199-205.
(157) Narita N, Narita M, Takashima S, Nakayama M, Nagai T, Okado N. Serotonin transporter gene variation is a risk factor for sudden infant death syndrome in the Japanese population. Pediatrics 2001; 107(4):690-692.
(158) Weese-Mayer DE, Berry-Kravis EM, Maher BS, Silvestri JM, Curran ME, Marazita ML. Sudden infant death syndrome: association with a promoter polymorphism of the serotonin transporter gene. Am J Med Genet A 2003; 117(3):268-274.
(159) Rand CM, Berry-Kravis EM, Zhou L, Fan W, Weese-Mayer DE. Sudden infant death syndrome: rare mutation in the serotonin system FEV gene. Pediatr Res 2007; 62(2):180-182.
(160) Moon RY, Horne RS, Hauck FR. Sudden infant death syndrome. Lancet 2007; 370(9598):1578-1587.
(161) Wang DW, Desai RR, Crotti L, Arnestad M, Insolia R, Pedrazzini M et al. Cardiac sodium channel dysfunction in sudden infant death syndrome. Circulation 2007; 115(3):368-376.
(162) Van Norstrand DW, Valdivia CR, Tester DJ, Ueda K, London B, Makielski JC et al. Molecular and functional characterization of novel glycerol-3-phosphate dehydrogenase 1 like gene (GPD1-L) mutations in sudden infant death syndrome. Circulation 2007; 116(20):2253-2259.
(163) Opdal SH, Vege A, Arnestad M, Musse MA, Rognum TO. Mitochondrial tRNA genes and flanking regions in sudden infant death syndrome. Acta Paediatr 2007; 96(2):211-214.
(164) Wailoo MP, Westaway JA, Joseph D, Petersen SA, Davies T, Thompson JR. Overnight deep body temperature and urinary cortisol excretion in infants from economically deprived areas. Child Care Health Dev 2003; 29(6):473-480.
(165) Malaty HM, Haveman T, Graham DY, Fraley JK. Helicobacter pylori infection in asymptomatic children: impact of epidemiologic factors on accuracy of diagnostic tests. J Pediatr Gastroenterol Nutr 2002; 35(1):59-63.
(166) Lee NN, Chan YF, Davies DP, Lau E, Yip DC. Sudden infant death syndrome in Hong Kong: confirmation of low incidence. BMJ 1989; 298(6675):721.
(167) Several authors. Comments on: Kerr et al. An association between sudden infant death syndrome (SIDS) and Helicobacter pylori infection. Arch Dis Child 2001; 84(6):525.
(168) Loddenkotter B, Becker K, Hohoff C, Brinkmann B, Bajanowski T. Real-time quantitative PCR assay for the detection of Helicobacter pylori: no association with sudden infant death syndrome. Int J Legal Med 2005; 119(4):202-206.
(169) Dubos F, Gottrand F, Devisme L, Carpentier C. The end of Helicobacter pylori as a hypothetic cause of SIDS. Am J Gastroenterol 2003; 98(5):1206-1207.
(170) Konstantopoulos N, Russmann H, Tasch C, Sauerwald T, Demmelmair H, Autenrieth I et al. Evaluation of the Helicobacter pylori stool antigen test (HpSA) for detection of Helicobacter pylori infection in children. Am J Gastroenterol 2001; 96(3):677-683.
(171) Kabir S. Review article: clinic-based testing for Helicobacter pylori infection by enzyme immunoassay of faeces, urine and saliva. Aliment Pharmacol Ther 2003; 17(11):1345-1354.
(172) Malfertheiner P, Megraud F, O'Morain C, Bazzoli F, El-Omar E, Graham D et al. Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report. Gut 2007; 56(6):772-781.
(173) Kolho KL, Klemola T, Koivusalo A, Rautelin H. Stool antigen tests for the detection of Helicobacter pylori in children. Diagn Microbiol Infect Dis 2006; 55(4):269-273.
(174) Fujimura S, Kato S, Nagai K, Kawamura T, Iinuma K. Detection of Helicobacter pylori in the stools of newborn infants. Pediatr Infect Dis J 2004; 23(11):1055-1056.
(175) Mandar R, Mikelsaar M. Transmission of mother's microflora to the newborn at birth. Biol Neonate 1996; 69(1):30-35.
(176) Palmer C, Bik EM, Digiulio DB, Relman DA, Brown PO. Development of the Human Infant Intestinal Microbiota. PLoS Biol 2007; 5(7):e177.
(177) Hveem K, Krüger O. The prevalence and transmission of Helicobacter Pylori in a large population based screening programme in Norway. The Nord-Trøndelag healthstudy (HUNT). EHSG: XVIth International Workshop in Gastrointestinal Pathology and Helicobacter 2003;386-387.

(178) Cevrioglu AS, Altindis M, Yilmazer M, Fenkci IV, Ellidokuz E, Kose S. Efficient and non-invasive method for investigating Helicobacter pylori in gravida with hyperemesis gravidarum: Helicobacter pylori stool antigen test. J Obstet Gynaecol Res 2004; 30(2):136-141.
(179) Delport W, van der Merwe SW. The transmission of Helicobacter pylori: the effects of analysis method and study population on inference. Best Pract Res Clin Gastroenterol 2007; 21(2):215-236.
(180) Adams BL, Bates TC, Oliver JD. Survival of Helicobacter pylori in a natural freshwater environment. Appl Environ Microbiol 2003; 69(12):7462-7466.
(181) Opdal SH, Rognum TO. The sudden infant death syndrome gene: does it exist? Pediatrics 2004; 114(4):e506-e512.
(182) Khamri W, Worku ML, Anderson AE, Walker MM, Hawgood S, Reid KB et al. Helicobacter infection in the surfactant protein D-deficient mouse. Helicobacter 2007; 12(2):112-123.

Errata

1. In the original manuscript, a system error had occurred in the Reference manager software program prior to printing. All in-text reference codes in the general discussion had shifted one paragraph up; i.e. the reference code at the end of one paragraph belonged to the subsequent paragraph with reference code.
2. (p8) Deleted misprint *"the of the".*
3. (p8) *"Dr. Shelly Tonkin"* corrected to *"Dr. Shirley Tonkin"*
4. (p11) *"the relative percentage of explained deaths have increased"* corrected to *"…has increased"*
5. (p11) "deaths scene investigation" corrected to "death scene investigation"
6. (p13) *"seem"* corrected to *"seems"*
7. (p14) *"infectious deaths"* corrected to *"deaths due to infections"*
8. (p15) "a factor in SIDS" changed to "contributory factors to SIDS"
9. (p16) *"gene polymophisms was"* corrected to *"…were"*
10. (p16) *"SIDS took an intermediate position indicating that IL-10 behaves like in infectious deaths in a proportion of the SIDS victims"* changed to *"…intermediate position between deaths due to infections and controls."*
11. (p17) *"the prevalence in children is low"* changed to *"…is presumably low"*
12. (p17) *"flagella"* corrected to *"flagellae"*
13. (p18) *"still not fully understood"* changed to "*yet incompletely understood"*
14. (p19) *"they"* changed to *"these"*
15. (p19) *"SP-B and SP-C are small hydrophobic…"* moved down one paragraph.
16. (p19) the phrase *"…members of the collectin family of proteins*" deleted
17. (p21) *"One aim"* changed to *"The aim"*
18. (p23) *"Rikshosptialet*" corrected to *"Rikshospitalet"*
19. (p23) "haplotypes" changed to *"haplotyping"*
20. (p31) *"anemia"* corrected to *"anaemia"*
21. (p31) *"some external or internal factor as a trigger"* changed to *"trigger – some external or…"*
22. (p31) "of occurrence of SIDS" changed to "of SIDS occurrence"
23. (p31) corrected misprints in the following words: *"researchers", "in", "stated", "genome"*
24. (p32) *"were also recently been reported"* changed to *"was recently reported"*
25. (p32) *"developmental*" corrected to *"developmental"*
26. (p33) *"Most frequently, infants…"* changed to "*The vast majority of infants…"*
27. (p35) *"finding"* corrected to *"findings"* and the phrase *"…shown to be"* deleted
28. (p36) *"from age-matched controls"* changed to *"from the age-matched live controls"*
29. *(p37)* Figure 7 missing in original manuscript
30. (p38) *"normal health individuals"* changed to *"individuals of normal health"*
31. (p39) *"showed"* changed to *"show"* and *"present"*
32. (p39) *"We showed in paper III"* changed to *"In paper III we present"*
33. (p40) *"Microarray"* changed to *"Real-time PCR"* and *"gene-sequencing"* deleted